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(54) Title: PERIPHERAL NERVOUS SYSTEM SPECIFIC SODIUM CHANNELS, DNA ENCODING THEREFOR, CRYSTALLIZATION, X-RAY DIFFRACTION, COMPUTER MOLECULAR MODELING, RATIONAL DRUG DESIGN, DRUG SCREENING, AND METHODS OF MAKING AND USING THEREOF			
(57) Abstract  Cloning, expression, viral and delivery vectors and hosts which contain nucleic acid coding for at least one peripheral nervous system specific (PNS) sodium channel peptide (SCP), isolated PNS SCP, and compounds and compositions and methods, are provided, for isolating, crystallizing, x-ray analysing molecular modeling, rational drug designing, selecting, making and using therapeutic or diagnostic agents or ligands having at least one peripheral nervous system specific (PNS) sodium channel (SC) modulating activity.			

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***Peripheral Nervous System Specific Sodium Channels,  
DNA Encoding Therefor, Crystallization, X-Ray Diffraction, Computer Molecular Modeling,  
Rational Drug Design, Drug Screening, and Methods of Making and Using Thereof***

***Cross-Reference to Related Applications***

- 5 This application is a continuation-in-part of U.S. Application No. 08/482,401, filed June 7, 1995, which is a continuation-in-part of U.S. Application No. 08/334,029 filed November 2, 1994, both of which disclosures are entirely incorporated herein by reference

***Statement as to Rights to Inventions Made Under Federally-Sponsored Research and Development***

- 10 The present invention was made with U.S. government support. Therefore, the U.S. government has certain rights in the invention.

***Field of the Invention***

- 15 The present invention is in the fields of biotechnology, protein purification and crystallization, x-ray diffraction analysis, three-dimensional computer molecular modeling, and rational drug design (RDD). The invention is directed to isolated peripheral nervous system (PNS) specific sodium channel proteins (SCPs) and encoding nucleic acid, as well as to compounds, compositions and methods for selecting, making and using therapeutic or diagnostic agents having sodium channel modulating activity. The present invention further provides three-dimensional computer modeling of the PNS SCP, and for RDD, based on the use of x-ray data and/or amino acid sequence data on computer readable media.

***Background of the Invention***

- 20 Voltage-sensitive ion channels are a class of transmembrane proteins that provide a basis for cellular excitability, as the ability to transmit information via ion-generated membrane potentials. In response to changes in membrane potentials, these molecules mediate rapid ion flux through highly selective pores in a nerve cell membrane. If the channel density is high enough, a suitable regenerative depolarization results, termed the action potential.

- 25 The voltage-sensitive sodium channel is the ion channel most often responsible for generating the action potential in excitable cells. Although sodium-based action potentials in different excitable tissues look similar (Hille, B., In: *Ionic Channels of Excitable Membranes*, B. Hille, ed., Sinauer, Sunderland, MA, (1984), pp. 70-71) recent electrophysiological studies indicate that sodium channels in different cells differ in both their structural and functional properties, and many sodium channels with distinct primary structures have now been identified. See, e.g., Mandel, *J. Membrane Biol.* 125:193-205 (1992).

- 30 Functionally distinct sodium channels have been described in a variety of neuronal cell types (Llinas *et al.*, *J. Physiol.* 305:197-213 (1980); Kostyuk *et al.*, *Neuroscience* 6:2423-2430 (1981); Bossu *et al.*, *Neurosci. Lett.* 51:241-246 (1984) 1981; Gilly *et al.*, *Nature* 309:448-450 (1984); French *et al.*, *Neurosci. Lett.* 56:289-294 (1985); Ikeda *et al.*, *J. Neurophysiol.* 55:527-539 (1986); Jones *et al.*, *J. Physiol.* 389:605-627 (1987); Alonso & Llinas, 1989; Gilly *et al.*, *J. Neurosci.* 9:1362-1374 (1989)) and in skeletal muscle (Gonoi *et al.*, *J. Neurosci.* 5:2559-2564 (1985); Weiss *et al.*, *Science* 233:361-364 (1986)). The kinetics of sodium currents in glia and neurons can also be distinguished (Barres *et al.*, *Neuron* 2:1375-1388 (1989)).

The type II and type III genes, expressed widely in the central nervous system (CNS), are expressed at very low levels in some cells in the PNS (Beckh, S., *FEBS Lett.* 262:317-322 (1990)). The type II and III mRNAs were barely detectable, by Northern blot analysis, in dorsal root ganglion (DRG), cranial nerves and sciatic nerves. On the

other hand, type I mRNA was present in moderately high amounts in DRG and cranial nerve, but in low levels in sciatic nerve. A comparison of the amount of all three brain mRNAs, relative to total sodium channel mRNA detected with a conserved cDNA probe, suggested the presence of additional, as yet unidentified, sodium channel types in DRG neurons. Consistent with the mRNA studies, immunochemical studies showed that neither type I nor type II sodium channel alpha subunits made up a significant component of the total sodium channels in the superior cervical ganglion or sciatic nerve (Gordon *et al.*, *Proc. Natl. Acad. Sci. USA* 84:8682-8686 (1987)).

A population of neurons in vertebrate DRG has been identified electrophysiologically that contains, in addition to the more conventional channels, a distinct sodium channel type; this DRG channel has a  $k_o$  for TTX approximately tenfold higher than the  $k_o$  of sodium channels in either skeletal muscle or heart (Jones *et al.*, *J. Physiol.* 389:605-627 (1987)).

The localization of different sodium channels to specific regions in the nervous system supports the possibility that cell-specific regulation of this gene family is at the transcriptional level. By analogy with other eukaryotic genes, distinct DNA elements can be present which mediate cell-specific and temporal regulation of individual sodium channel genes.

Studies of sodium channel gene regulation have been facilitated by the use of well-characterized cell lines, such as pheochromocytoma (PC12) cells, a popular cell model for neuronal differentiation (Green *et al.*, *Proc. Natl. Acad. Sci. USA* 73:2424-2428 (1976); Halegoua *et al.*, *Curr. Top. Microbiol. Immunol.* 165:119-170 (1991)). In addition to extending neurites and initiating synthesis of certain neurotransmitters, NGF-treated PC12 cells acquire the ability to generate sodium-based action potentials (Dichter *et al.*, *Nature* 268:501-504 (1977)). This ability is conferred by an increase in the density of functional sodium channels in the membranes of the NGF-treated cells (Rudy *et al.*, *J. Neurosci.* 7:1613-1625 (1987); Mandel *et al.*, *Proc. Natl. Acad. Sci. USA* 85:924-928 (1988); O'Laugh *et al.*, *Proc. Natl. Acad. Sci. USA* 77:1701-1705 (1980)). Northern blot analysis revealed that undifferentiated PC12 cells contained a basal level of sodium channel mRNA which increased coincident with the increase in channel activity observed after treatment with NGF (Mandel *et al.*, *Proc. Natl. Acad. Sci. USA* 85:924-928 (1988)).

There is a long standing need to diagnose and/or treat pathologies relating to impaired peripheral nervous system (PNS) nerve conduction associated with PNS injury or in genetic or other disease states, such as those involving lack of, or defects in, PNS sodium channels (SCs). In view of the possibility of cell or tissue specific sodium channels, the discovery and use of isolated PNS SCs and encoding nucleic acid would provide an opportunity to diagnose or treat such pathologies by either screening suitable PNS SC modulating drugs or molecules (e.g., analgesics), or by using recombinant PNS SCs for *in situ* or *in vivo* gene therapy to replace or supplement PNS SCs in at least one portion of the peripheral nervous system of a mammalian patient suffering from a PNS SC related pathology.

### Summary of the Invention

The present invention (hereinafter, "invention") provides peripheral nervous system specific (PNS) sodium channel peptides (SCPs), encoding nucleic acid, vectors, host cells and antibodies, as well as methods of making and using thereof, including recombinant expression, purification, cell-based drug screening, gene therapy, crystallization, X-ray diffraction analysis, as well as computer structure determination and rational drug design utilizing at least one PNS SCP amino acid sequence and/or x-ray diffraction data provided on computer readable media.

The invention also includes oligonucleotide probes specific for PNS SCP encoding sequences, as well as methods for detection in a sample, where the probe is labeled. The invention further includes methods for producing a PNS SCP, comprising culturing a host in a culture medium, comprising a PNS SCP nucleic acid; and isolating the PNS SCP from said host or said culture medium.

5 The invention additionally includes an antibody which binds an epitope specific for a PNS SCP, as well as host cells which express the antibody. Diagnostic or therapeutic methods using the antibody are also included in the invention.

The invention further includes gene therapy methods and delivery vectors comprising nucleic acid encoding, or complementary to, at least one PNS SCP, and pharmaceutically acceptable compositions thereof.

10 The invention also includes gene therapy by methods that administer an antisense PNS SCP nucleic acid to an animal in amount effective to provide a PNS SC modulating effect, such as an analgesic effect.

The present invention further provides methods for purifying and crystallizing a PNS SCP that can be analyzed to obtain x-ray diffraction patterns of sufficiently high resolution to be useful for three-dimensional molecular modeling of the protein. The x-ray diffraction data, atomic coordinates, and/or amino acid sequences provided on computer readable medium, are modeled on computer systems, using methods of the invention, to generate secondary, tertiary, 15 and/or quaternary structures of a PNS SCP, which structures contribute to their overall three dimensional structure, as well as binding and active sites of the PNS SCP.

Molecular modeling methods and computer systems are also provided by the present invention for rational drug design (RDD). These drug design methods use computer modeling programs to find potential ligands or agents that 20 are calculated to bind with sites or domains on the PNS SCP. Potential ligands or agents are then screened for modulating or binding activity. Such screening methods can be selected from assays for at least one biological activity of the protein, as associated with a PNS SCP-related pathology or trauma, according to known sodium channel assays. The resulting ligands provided by methods of the present invention are synthesized and are useful for treating, inhibiting or preventing at least one of PCS SCP-related pathology or trauma in a mammal.

25 Further objects, features, utilities, embodiments and/or advantages of the present invention will be apparent from the additional description provided herein.

### ***Brief Description of the Drawings***

*Figure 1* depicts a 323 amino acid and corresponding 969 nucleotide sequence of a PNS SCP as amino acids 233-555 of SEQ ID NO:2 and nucleotides 699-1665 of SEQ ID NO:1, as the primary structure of Domain III of the 30 Peripheral Nerve type I (PNI) sodium channel alpha () subunit for both amino acid and DNA sequences. The single amino acid code is used to denote deduced amino acids. YJ1 and YOIC refer to the oligonucleotide primers used to obtain the initial PCR fragment of PNI cDNA.

*Figure 2A-B* shows a Northern blot analysis of sodium channel  $\alpha$  subunit mRNA in rat pheochromocytoma (PC12) cells treated with Nerve Growth Factor. In *Figure 2(A)*, the probe used is pRB211 which encodes the highly 35 conserved fourth repeated domain of the rat type II sodium channel. Both type II and PNI mRNAs are detected with this probe. In *Figure 2(B)*, the probe used contains sequences specific for PNI. The levels of sodium channel mRNA

are quantitated with reference to the amount of cyclophilin mRNA, as indicated. Control cells are PC 12 cells grown in the absence of NGF.

**Figure 3A-B** shows an example of tissue-specific distribution of PN1 mRNA. Figure 3(A) presents a Northern blot analysis using equal amounts of RNA from tissues. PN1 mRNA is indicated by the dash. 28S refers to the 28S rRNA. The probe contains sequences specific for the PN1 gene. Note the absence of PN1 mRNA in skeletal muscle, cardiac muscle, and the low levels of PN1 mRNA in spinal cord. Figure 3(B) shows RNAase protection analysis of PN1 mRNA. PN1 refers to the PN1 probe protected by mRNA from the different tissue samples. Actin refers to actin probe sequences protected by the same mRNA.

**Figure 4A-F** shows localization of PN1 mRNA in Superior Cervical Ganglion (SCG) and Dorsal Root Ganglion (DRG) tissues by *in situ* hybridization analysis. Figures 4A-4B represent neurons hybridized with a PN1-specific antisense RNA probe. Figures 4C-4D represent neurons hybridized with the radiolabeled PN1 probe in the presence of non-labeled PN1 competitor DNA. Figures 4E-4F represent tissue sections hybridized with an antisense type II probe.

**Figure 5** shows a blot analysis comparing Levels of PN1 and brain type I a subunit mRNA in SCG. The pRB11 conserved sodium channel probe detects both type II/IIA and PN1 transcripts.

**Figure 6A-B** shows a Northern blot analysis which reveals differential expression of PN1 and type I sodium channel mRNAs during postnatal rat development. Figure 6(A) shows a representative autoradiogram of a Northern blot using radiolabeled antisense pRB211 RNA as probe. Postnatal days 7 (P7) to 42 (P42) are shown. Figure 6(B) shows a plot of quantitation of the Northern blots showing a decrease in type I mRNA with time after birth.

**Figure 7A-D** show the deduced primary structure of cloned portion of PN1 a subunit cDNA as a partial 3033 nucleotide (SEQ ID NO:1) sequence and a partial 1011 amino acid (SEQ ID NO:2) sequence.

**Figure 8A-D** show a comparison of deduced primary amino acid sequences of PN1 (1-988 of SEQ ID NO:2) and brain type II/IIA  $\alpha$  subunit (SEQ ID NO:3).

**Figure 9A-9D** show the entire DNA sequence for a rat PN1 PNS SCP (SEQ ID NO:9).

**Figure 10** shows the entire amino sequence for a rat PN1 PNS SCP (SEQ ID NO:10).

**Figure 11A-11E** shows amino acid sequences for rat PN1 ("RATPN1") (SEQ ID NO:10) and two expected human PN1 sequences "HUMPN1A" (SEQ ID NO:11) "HUMPN1B" (SEQ ID NO:12) HUMPN1C (SEQ ID NO:7) and HUMPN1D (SEQ ID NO:3). Alternative sequences include those where "X" is 0, 1, 2, or 3 of the same or different amino acids, which can be optionally selected from Table 1 or Table 2.

**Figure 12** shows a computer system suitable for three dimensional structure determination and/or rational drug design.

**Figure 13A-B** shows a representative DNA sequence encoding a human PN1 (HUM PN1A) (SEQ ID NO:11)

**Figure 14-B** shows a representative DNA sequence encoding a human PN1 (HUM PN1B) (SEQ ID NO:12)

### Detailed Description of the Invention

A need exists for modulating the activity of at least one peripheral nervous system specific (PNS) sodium channel (SCs). Such modulation could potentially provide analgesic or diagnostic agents for pain or pathologies associated with nerve conduction in the PNS.

- 5 Certain sodium channels --corresponding to PNS SCPs of the invention-- are now discovered to be preferentially or selectively expressed in the peripheral nervous system (PNS). These sodium channels modulate peripheral nerve impulse conduction preferentially in the PNS. The present invention provides peripheral nervous system specific (PNS) sodium channel peptides (SCPs), encoding nucleic acid, vectors, host cells and antibodies, as well as methods of making and using thereof, including recombinant expression, purification, cell-based drug screening, gene
- 10 therapy, crystallization, X-ray diffraction analysis, as well as computer structure determination and rational drug design utilizing at least one PNS SCP amino acid sequence and/or x-ray diffraction data provided on computer readable media.

A PNS sodium channel peptide (PNS SCP) can refer to any subset of a PNS sodium channel (SC) having SC activity, as a fragment, consensus sequence or repeating unit. A PNS SCP of the invention can be prepared by:

- 15 (a) recombinant DNA methods;
- (b) proteolytic digestion of the intact molecule or a fragment thereof;
- (c) chemical peptide synthesis methods well-known in the art; and/or
- (d) by any other method capable of producing a PNS SCP and having a conformation similar to an active portion of a PNS SCP and having SC activity. The SC activity can be screened according to known screening assays for sodium channel activity, *in vitro*, *in situ* or *in vivo*. The minimum peptide sequence to have activity is based on the
- 20 smallest unit containing or comprising a particular region, domain, consensus sequence, or repeating unit thereof, of at least one PNS SCP.

- According to the invention, a PNS SCP includes an association of two or more polypeptide domains, such as transmembrane, pore lining domains, or fragments thereof, corresponding to a PNS SCP, such as I-40 domains or any range or value therein. Transmembrane, cytoplasmic pore lining or other domains of a PNS SCP of the invention may
- 25 have at least 74% homology, such as 74-100% overall homology or identity, or any range or value therein to one or more corresponding SC domains as described herein (*e.g.*, as presented Figures 1, 7, 8, 10 or 11). As would be understood by one of ordinary skill in the art, the above configuration of domains are provided as part of a PNS SCP of the invention, such that a functional PNS SCP, when expressed in a suitable cell, is capable of transporting sodium ions across a lipid bilayer, a cell membrane or a membrane model. In intact cells having sufficient sodium channels,
- 30 the cell can be capable of generating some form of an action potential, such as in a cell expressing at least one PNS SCP of the present invention. Such transport, as measured by suitable SC activity assays, establishes SC activity of one or more PNS SCPs of the invention.

- Accordingly, a PNS SCP of the invention alternatively includes peptides having a portion of a SC amino acid sequence which substantially corresponds to at least one 20 to 2005 amino acid fragment and/or consensus sequence
- 35 of a PNS SCP or group of PNS SCPs, wherein the PNS SCP has homology or identity of at least 74-99%, such as 88-99% (or any range or value therein, *e.g.*, 87-99, 88-99, 89-99, 90-99, 91-99, 92-99, 93-99, 94-99, 95-99, 96-99, 97-99, or 98-99%) homology to at least one sequence or consensus sequence of Figures 1, 7, 8, 10 or 11. In one aspect, such

a PNS SCP can maintain SC biological activity. It is preferred that a PNS SCP of the invention is not naturally occurring or is naturally occurring but is in a purified or isolated form which does not occur in nature. Preferably, a PNS SCP of the invention substantially corresponds to an set of domains of PN1, having at least 10 contiguous amino acids of Figures 1, 7, 8, 10 and 11, or at least 74% homology thereto.

Alternatively or additionally, a PNS SCP of the invention may comprise at least one domain corresponding to known sodium channel domains, such as rat brain or spinal cord SC domains, such as transmembrane domains, pore lining domains, cytoplasmic domains or extracellular domains, such as IIs6 (e.g., 1-3 to 14-17 (IIs6), 18-23 to 210-214 (cytoplasmic), 229-236 to 254-258 (IIs1), 268-272 to 293-297 (IIs2), 300-304 to 321-325 (IIs3), 326-330 to 347-351 (IIs4), 368-374 to 389-393 (IIs5), 474-478 to 500-504 (IIs6), 553-559 to 577-583 (IVs1), 589-593 to 611-615 (IVs2), 619-623 to 642-646 (IVs3), 654-658 to 678-682 (IVs4), 690-694 to 711-715 (IVs5), 779-783 to 801-805 (IVs6), 348-352 to 368-372, 501-505 to 550-554, 233-555, 676-678 to 689-693, 554-557 to 941-945, or any range or value therein, corresponding to SEQ ID NO:2 as presented in Figure 7A-7D, or variants thereof as presented substitutions in Table 1 or Table 2, having 74-100% overall homology or any range or value therein. At least one of such domains are present in the PNS SCPs presented in Figure 11A-E, or fragments thereof, as non-limiting examples. Alternative domains are also encoded by DNA which hybridizes under stringent conditions to at least 30 contiguous nucleotides of Figures 1, 7, 9, 13 or 14, or having codons substituted therefor which encode the same amino acid as a particular codon. Additionally, phosphorylation (e.g., PKA and PKC) domains, as would be recognized by the those skilled in the art are also considered when providing a PNS SCP or encoding nucleic acid according to the invention.

Percent homology or identity can be determined, for example, by comparing sequence information using the GAP computer program, version 6.0, available from the University of Wisconsin Genetics Computer Group (UWGGC). The GAP program utilizes the alignment method of Needleman and Wunsch (*J. Mol. Biol.* 48:443 (1970), as revised by Smith and Waterman (*Adv. Appl. Math.* 2:482 (1981)). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) which are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program include: (1) a unitary comparison matrix (containing a value of 1 for identities and 0 for non-identities) and the weighted comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745 (1986), as described by Schwartz and Dayhoff, eds., *ATLAS OF PROTEIN SEQUENCE AND STRUCTURE*, National Biomedical Research Foundation, pp. 353-358 (1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. In a preferred embodiment, the peptide of the invention corresponds to a SC biologically active portion of SEQ ID NO:2, or variant thereof, e.g., as presented in Figure 11A-D.

Thus, one of ordinary skill in the art, given the teachings and guidance presented in the present specification, will know how to add, delete or substitute other amino acid residues in other positions of a SC to obtain a PNS SCP, including substituted, deletional or additional variants, e.g., with a substitution as presented in Tables 1 or 2 below.

A PNS SCP of the invention also includes a variant wherein at least one amino acid residue in the peptide has been conservatively replaced, added or deleted by at least one different amino acid. For a detailed description of protein chemistry and structure, see, e.g., Schulz, et al., *Principles of Protein Structure*, Springer-Verlag, New York, 1978, and Creighton, T.E., *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, 1983, which are hereby incorporated by reference. For a presentation of nucleotide sequence substitutions, such as codon



preferences, see Ausubel *et al.*, eds, *Current Protocols in Molecular Biology*, Greene Publishing Assoc., New York, NY (1987, 1992, 1993, 1994, 1995) at §§ A.1.1-A.1.24, and Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), at Appendices C and D.

- Conservative substitutions of a PNS SCP of the invention includes a variant wherein at least one amino acid residue in the peptide has been conservatively replaced, added or deleted by at least one different amino acid. Such substitutions preferably are made in accordance with the following list as presented in Table 1, which substitutions can be determined by routine experimentation to provide modified structural and functional properties of a synthesized peptide molecule, while maintaining SC biological activity, as determined by known SC activity assays. In the context of the invention, the term PNS SCP or "substantially corresponding to" includes such substitutions.

Table 1

Original Residue	Exemplary Substitution
Ala	Gly; Ser
Arg	Lys
Asn	Gln; His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Ala; Pro
His	Asn; Gln
Ile	Leu; Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Tyr; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

Alternatively, another group of substitutions of PNS SCPs of the invention are those in which at least one amino acid residue in the protein molecule has been removed and a different residue added in its place according to the following Table 2. The types of substitutions which can be made in the protein or peptide molecule of the invention can be based on analysis of the frequencies of amino acid changes between a homologous protein of different species, such as those presented in Table 1-2 of Schulz *et al.*, *infra*. Based on such an analysis, alternative conservative substitutions are defined herein as exchanges within one of the following five groups:

TABLE 2

1. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr (Pro, Gly);
2. Polar, negatively charged residues and their amides: Asp, Asn, Glu, Gln;
3. Polar, positively charged residues: His, Arg, Lys;
4. Large aliphatic, nonpolar residues: Met, Leu, Ile, Val (Cys); and
5. Large aromatic residues: Phe, Tyr, Trp.

Most deletions and additions, and substitutions according to the invention are those which do not produce radical changes in the characteristics of the protein or peptide molecule. "Characteristics" is defined in a non-inclusive manner to define both changes in secondary structure, e.g.  $\alpha$ -helix or  $\beta$ -sheet, as well as changes in physiological activity, e.g. in receptor binding assays.

Accordingly, based on the above examples of specific substitutions, alternative substitutions can be made by routine experimentation, to provide alternative PNS SCPs of the invention, e.g., by making one or more conservative substitutions of SC fragments which provide SC activity. However, when the exact effect of the substitution, deletion, or addition is to be confirmed, one skilled in the art will appreciate that the effect of at least one substitution, addition or deletion will be evaluated by at least one sodium channel activity screening assay, such as, but not limited to, immunoassays or bioassays, to confirm biological activity, such as, but not limited to, sodium channel activity.

Amino acid sequence variants of a PNS SCP of the invention can also be prepared by mutations in the DNA. Such variants include, for example, deletions from, or additions or substitutions of, residues within the amino acid sequence. Any combination of deletion, addition, and substitution can also be made to arrive at the final construct, provided that the final construct possesses some SC activity. Preferably improved SC activity is found over that of the non-variant peptide. Obviously, mutations that will be made in the DNA encoding the variant must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure (see, e.g., EP Patent Application Publication No. 75,444; Ausubel, *infra*; Sambrook, *infra*). At the genetic level, these variants ordinarily are prepared by site-directed mutagenesis of nucleotides in the DNA encoding a PNS SCP, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. The variants typically exhibit the same qualitative biological activity as the naturally occurring SC (see, e.g., Ausubel, *infra*; Sambrook, *infra*).

Once a PNS sodium channel structure or characteristics have been determined, PNS SCPs can be recombinantly or synthetically produced, or optionally purified, to provide commercially useful amounts of PNS SCPs for use in diagnostic or research applications, according to known method steps (see, e.g., Ausubel, *infra*, and Sambrook, *infra*, which references are herein entirely incorporated by reference).

A variety of methodologies known in the art can be utilized to obtain an isolated PNS SCP of the invention. In one embodiment, the peptide is purified from tissues or cells which naturally produce the peptide. Alternatively, the above-described isolated nucleic acid fragments could be used to express the PNS SCP protein in any organism. The samples of the invention include cells, protein extracts or membrane extracts of cells, or biological fluids. The sample will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts used as the sample.

The cells and/or tissue can include, e.g., normal or pathologic animal cells or tissues, such as the peripheral nervous system, and extracts or cell cultures thereof, provided *in vivo*, *in situ* or *in vitro*, as cultured, passaged, non-passaged, transformed, recombinant, or isolated cells and/or tissues.

Any higher eukaryotic organism can be used as a source of at least one PNS SCI or PNS SCP of the invention, as long as the source organism naturally contains such a peptide. As used herein, "source organism" refers to the original organism from which the amino acid sequence of the peptide is derived, regardless of the organism the peptide is expressed in and/or ultimately isolated from. Preferred organisms as sources of at least one PNS SCI or encoding

nucleic acid can be any vertebrate animal, such as mammals, birds, bony fish, electric eels, frogs and toads. Among mammals, the preferred recipients are mammals of the Orders Primata (including humans, apes and monkeys), Arteriodactyla (including horses, goats, cows, sheep, pigs), Rodenta (including mice, rats, rabbits, and hamsters), and Carnivora (including cats, and dogs). The most preferred source organisms are humans.

One skilled in the art can readily follow known methods for isolating proteins in order to obtain the peptide free of natural contaminants. These include, but are not limited to: immunochromatography, size-exclusion chromatography, HPLC, ion-exchange chromatography, and immunoaffinity chromatography. See, e.g., Ausubel, *infra*; Sambrook, *infra*; Colligan, *infra*.

**Isolated Nucleic Acid Molecules Coding for PNS SCP Peptides** In one embodiment, the present invention relates to an isolated nucleic acid molecule coding for a peptide having an amino acid sequence corresponding to novel PNS SCPs. In one preferred embodiment, the isolated nucleic acid molecule comprises a PNS SCP nucleotide sequence with greater than 70% overall identity or homology to at least a 60 nucleotide sequence present in SEQ ID NO:1 (preferably greater than 80%; more preferably greater than 90%, such as 70-99% any range or value therein). In another preferred embodiment, the isolated nucleic acid molecule comprises a PNS SCP nucleotide sequence corresponding to Figures 1, 7 or 9, or encoding at least one domain of Figures 1, 7, 8, 10 and 11.

Also included within the scope of this invention are the functional equivalents of the herein-described isolated nucleic acid molecules and derivatives thereof. For example, as presented above for PNS SCP amino acid sequences, the nucleic acid sequences depicted in SEQ ID NO:1 can be altered by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence of a PNS SCP can be used in the practice of the invention. These include but are not limited to amino acid sequences encoding all or portions of PNS SCP amino acid sequence of Figures 1, 8, 10 and 11, which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change.

Such functional alterations of a given nucleic acid sequence afford an opportunity to promote secretion and/or processing of heterologous proteins encoded by foreign nucleic acid sequences fused thereto. All variations of the nucleotide sequence of the PNS SCP gene and fragments thereof permitted by the genetic code are, therefore, included in this invention. See, e.g., Ausubel, *infra*; Sambrook, *infra*.

In addition, the nucleic acid sequence can comprise a nucleotide sequence which results from the addition, deletion or substitution of at least one nucleotide to the 5'-end and/or the 3'-end of a nucleic acid sequence corresponding to Figures 1, 7 or 9, or encoding at least a portion of Figures 1, 8, 10 or 11, or a variant thereof. Any nucleotide or polynucleotide can be used in this regard, provided that its addition, deletion or substitution does not remove the sodium channel activity which is encoded by the nucleotide sequence. Moreover, the nucleic acid molecule of the invention can, as necessary, have restriction endonuclease recognition sites which do not remove the activity of the encoded PNS SCP.

Further, it is possible to delete codons or to substitute one or more codons by codons other than degenerate codons to produce a structurally modified peptide, but one which has substantially the same utility or activity of the peptide produced by the unmodified nucleic acid molecule. As recognized in the art, the two peptides are functionally equivalent, as are the two nucleic acid molecules which give rise to their production, even though the differences

between the nucleic acid molecules are not related to degeneracy of the genetic code. See, e.g., Ausubel, *infra*; Sambrook, *infra*.

**Isolation of Nucleic Acid** In another aspect of the present invention, isolated nucleic acid molecules coding for peptides having amino acid sequences corresponding to PNS SCP are provided. In particular, the nucleic acid molecule can be isolated from a biological sample containing mammalian nucleic acid, as corresponding to a probe specific for a PNS SC obtained from a higher eukaryotic organism.

The nucleic acid molecule can be isolated from a biological sample containing nucleic acid using known techniques, such as but not limited to, primer amplification or cDNA cloning.

The nucleic acid molecule can be isolated from a biological sample containing genomic DNA or from a genomic library. Suitable biological samples include, but are not limited to, normal or pathologic animal cells or tissues, such as cerebrospinal fluid (CNS), peripheral nervous system (neurons, ganglion) and portions, cells of heart, smooth, skeletal or cardiac muscle, autonomic nervous system, and extracts or cell cultures thereof, provided *in vivo*, *in situ* or *in vitro*, as cultured, passaged, non-passaged, transformed, recombinant, or isolated cells and/or tissues. The method of obtaining the biological sample will vary depending upon the nature of the sample.

One skilled in the art will realize that a mammalian genome can be subject to slight allelic variations between individuals. Therefore, the isolated nucleic acid molecule is also intended to include allelic variations, so long as the sequence encodes a PNS SCP. When a PNS SCP allele does not encode the identical amino acid sequence to that found in Figures 1, 8, 10 or 11, or at least domain thereof, it can be isolated and identified as PNS SCP using the same techniques used herein, and especially nucleic acid amplification techniques to amplify the appropriate gene with primers based on the sequences disclosed herein. Such variations are presented, e.g., in Figure 11 and in Tables 1 and 2..

The cloning of large cDNAs is the same (e.g., PN1 as a PNS SCP of the invention includes overlapping clones of about 13kDa) but takes more routine experimentation, than smaller cDNAs. One useful method relies on cDNA bacteriophage library screening (see, e.g., Sambrook, *infra*, or Ausubel, *infra*). Probes for the screening are labeled, e.g., with random hexamers and Klenow enzyme (Pharmacia kit). If 5' cDNAs are not obtained with these approaches, a subcDNA library can be prepared in which a specific PN1 primers are used to prime the reverse transcript reaction in place of oligo dT or random primers. The cDNA sublibrary is then cloned into standard vectors such as lambda zap and screened using conventional techniques. This strategy was used previously (Noda *et al.* *Nature* 320:188-192 (1986); Noda *et al.*, *Nature* 322:826-828 (1986)) to clone the brain type I and II sodium channel cDNAs. The construction of a full-length cDNA is performed by subcloning overlapping fragments into an expression vector (either prokaryotic or eukaryotic). This task is more difficult with large cDNAs because of the paucity of unique restriction sites, but routine restriction, cloning or PCR is used to join the fragments.

**Synthesis of Nucleic Acid** Isolated nucleic acid molecules of the present invention are also meant to include those chemically synthesized. For example, a nucleic acid molecule with the nucleotide sequence which codes for the expression product of a PNS SCP gene can be designed and, if necessary, divided into appropriate smaller fragments. Then an oligomer which corresponds to the nucleic acid molecule, or to each of the divided fragments, can be synthesized (e.g., of 10-6015 nucleotides or any range or value therein, such as 10-100 nucleotides). Such synthetic

oligonucleotides can be prepared, for example, by known techniques (See, e.g., Ausubel, *infra*, or Sambrook, *infra*) or by using an automated DNA synthesizer.

A labeled oligonucleotide probe be derived synthetically or by cloning. If necessary, the 5'-ends of the oligomers can be phosphorylated using T4 polynucleotide kinase. Kinasing of single strands prior to annealing or for labeling can be achieved using an excess of the enzyme. If kinasing is for the labeling of probe, the ATP can contain high specific activity radioisotopes. Then, the DNA oligomer can be subjected to annealing and ligation with T4 ligase or the like.

***A Nucleic Acid Probe for the Specific Detection of PNS SCP*** In another embodiment, the present invention relates to a nucleic acid probe of 15-6000 nucleotides for the specific detection of the presence of PNS SCP in a sample comprising the above-described nucleic acid molecules or at least a fragment thereof which binds under stringent conditions to a nucleic acid encoding at least one PNS SCP.

The nucleic acid probe can be used to screen an appropriate chromosomal or cDNA library by known hybridization method steps to obtain a PNS SCP encoding nucleic acid molecule of the invention. A chromosomal DNA or cDNA library can be prepared from appropriate cells according to recognized methods in the art (See, e.g., Ausubel, *infra*; Sambrook, *infra*).

In the alternative, organic chemical synthesis is carried out in order to obtain nucleic acid probes having nucleotide sequences which correspond to suitable portions of the amino acid sequence of the PNS SCP. Thus, the synthesized nucleic acid probes can be used as primers in nucleic acid amplification method steps.

The invention can thus provide methods for amplification of DNA and/or RNA using heat stable, cross-linked nucleotide primers, which cross linked primers of the invention to provide nucleic acid encoding PNS SCPs according to the invention.

Methods of amplification of RNA or DNA are well known in the art and can be used according to the invention without undue experimentation, based on the teaching and guidance presented herein. According to the invention, the use of nucleic acids encoding portions of PNS SCPs according to the invention, as amplification primers, allows for advantages over known amplification primers, due to the increase in sensitivity, selectivity and/or rate of amplification.

Known methods of DNA or RNA amplification include, but are not limited to polymerase chain reaction (PCR) and related amplification processes (see, e.g., U.S. patent Nos. 4,683,195, 4,683,202, 4,800,159, 4,965,188, to Mullis *et al.*; 4,795,699 and 4,921,794 to Tabor *et al.*; 5,142,033 to Innis; 5,122,464 to Wilson *et al.*; 5,091,310 to Innis; 5,066,584 to Gyllenstein *et al.*; 4,889,818 to Gelfand *et al.*; 4,994,370 to Silver *et al.*; 4,766,067 to Biswas; 4,656,134 to Ringold; 5,340,728 to Grosz *et al.*; 5,322,770 to Gelfand *et al.*; 5,338,671 to Scalice *et al.*; PCT WO 92/06200 to Cetus Corp.; PCT WO 94/14978 to Strack *et al.*, which patent disclosures are entirely incorporated herein by reference) and RNA mediated amplification which uses antisense RNA to the target sequence as a template for double stranded DNA synthesis (U.S. patent No. 5,130,238 to Malek *et al.*, with the tradename NASBA), the entire contents of which patents and references are herein entirely incorporated by reference. Reviews of the PCR are provided by Mullis (*Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986)); Saiki *et al.* (*BioTechnology* 3:1008-1012 (1985)); and Mullis *et al.* (*Meth. Enzymol.* 155:335-350 (1987)). One skilled in the art can readily design such probes based on the sequence disclosed herein using methods such as computer alignment and sequence analysis known in the art. See, e.g., Ausubel, *infra*; Sambrook, *infra*.

The hybridization probes of the invention can be labeled by standard labeling techniques such as with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, chemiluminescence, and any other known and suitable labels. After hybridization, the probes can be visualized using known methods. The nucleic acid probes of the invention include RNA, as well as DNA probes, such probes being generated using techniques known in the art (See, e.g., Ausubel, *infra*; Sambrook, *infra*). In one embodiment of the above described method, a nucleic acid probe is immobilized on a solid support. Examples of such solid supports include, but are not limited to, plastics such as polycarbonate, complex carbohydrates such as agarose and SEPHAROSE, and acrylic resins, such as polyacrylamide and latex beads. Techniques for coupling nucleic acid probes to such solid supports are well known in the art (See, e.g., Ausubel, *infra*; Sambrook, *infra*).

The test samples suitable for nucleic acid probing methods of the invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The sample used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample which is compatible with the method utilized.

**Methods for Detecting The Presence of PNS SCP Encoding Nucleic Acid in a Biological Sample.** In another embodiment, the present invention relates to methods for detecting the presence of PNS SCP encoding nucleic acid in a sample. Such methods can comprise (a) contacting the sample with the above-described nucleic acid probe, under conditions such that hybridization occurs, and (b) detecting the presence of a labeled probe bound to the nucleic acid probe. One skilled in the art can select a suitable, labeled nucleic acid probe according to techniques known in the art as described above. Samples to be tested include, but are not limited to, RNA samples of mammalian tissue.

PNS SCP has been found to be expressed in peripheral nerve and dorsal root ganglion cells. Accordingly, PNS SCP probes can be used detect the presence of RNA from PN cells in such a biological sample. Further, altered expression levels of PNS SCP RNA in an individual, as compared to normal levels, can indicate the presence of disease. The PNS SCP probes can further be used to assay cellular activity in general and specifically in peripheral nervous system tissue.

**A Kit for Detecting the Presence of PNS SCP in a Sample.** In another embodiment, the present invention relates to a kit for detecting the presence of PNS SCP in a sample comprising at least one container having disposed therein the above-described nucleic acid probe. In a preferred embodiment, the kit further comprises other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabeled probes, enzymatic labeled probes (horse radish peroxidase, alkaline phosphatase), and affinity labeled probes (biotin, avidin, or streptavidin) (See, e.g., Ausubel, *infra*; Sambrook, *infra*).

A compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the probe or primers used in the assay, containers which contain wash reagents (such as phosphate buffered

saline, TRIS-buffers, and the like), and containers which contain the reagents used to detect the hybridized probe, bound antibody, amplified product, or the like.

One skilled in the art will readily recognize that the nucleic acid probes described in the invention can readily be incorporated into one of the established kit formats which are well known in the art.

5 **DNA Constructs Comprising a PNS SCP Nucleic Acid Molecule and Hosts Containing These Constructs.** A nucleic acid sequence encoding a PNS SCP of the invention can be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. Techniques for such manipulations are disclosed, e.g., by Ausubel *et al.*, *infra*, and are well known in the art.

10 A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene expression as PNS SCPs or Ab fragments in recoverable amounts. The precise nature of the regulatory regions needed for gene expression can vary from organism to organism, as is well known in the analogous art. See, e.g., Sambrook, *infra* and Ausubel *infra*.

15 The invention accordingly encompasses the expression of a PNS SCP, in either prokaryotic or eukaryotic cells, although eukaryotic expression is preferred.

20 Preferred hosts are bacterial or eukaryotic hosts including bacteria, yeast, insects, fungi, bird and mammalian cells either *in vivo*, or *in situ*, or host cells of mammalian, insect, bird or yeast origin. It is preferred that the mammalian cell or tissue is of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog or cat origin, but any other mammalian cell can be used.

25 Eukaryotic hosts can include yeast, insects, fungi, and mammalian cells either *in vivo*, or in tissue culture. Preferred eukaryotic hosts can also include, but are not limited to insect cells, mammalian cells either *in vivo*, or in tissue culture. Preferred mammalian cells include *Xenopus* oocytes, HeLa cells, cells of fibroblast origin such as VERO or CHO-K1, or cells of lymphoid origin and their derivatives.

30 Mammalian cells provide post-translational modifications to protein molecules including correct folding or glycosylation at correct sites. Mammalian cells which can be useful as hosts include cells of fibroblast origin such as, but not limited to, NIH 3T3, VERO or CHO, or cells of lymphoid origin, such as, but not limited to, the hybridoma SP2/O-Ag14 or the murine myeloma P3-X63Ag8, hamster cell lines (e.g., CHO-K1 and progenitors, e.g., CHO-DUXB11) and their derivatives. One preferred type of mammalian cells are cells which are intended to replace the function of the genetically deficient cells *in vivo*. Neuronally derived cells are preferred for gene therapy of disorders of the nervous system. For a mammalian cell host, many possible vector systems are available for the expression of at least one PNS SCP. A wide variety of transcriptional and translational regulatory sequences can be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals can be derived from viral sources, such as, but not limited to, adenovirus, bovine papilloma virus, Simian virus, or the like, where the regulatory signals are associated with a particular gene which has a high level of expression. Alternatively, promoters from

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mammalian expression products, such as, but not limited to, actin, collagen, myosin, protein production. See, Ausubel, *infra*; Sambrook, *infra*.

When live insects are to be used, silk moth caterpillars and baculoviral vectors are presently preferred hosts for large scale PNS SCP production according to the invention. Production of PNS SCPs in insects can be achieved, for example, by infecting the insect host with a baculovirus engineered to express at least one PNS SCP by methods known to those skilled in the related arts. See Ausubel *et al*, eds. *Current Protocols in Molecular Biology*, Wiley Interscience, §§16.8-16.11 (1987, 1992, 1993, 1994).

In a preferred embodiment, the introduced nucleotide sequence will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors can be employed for this purpose. See, *e.g.*, Ausubel *et al.*, *infra*, §§ 1.5, 1.10, 7.1, 7.3, 8.1, 9.6, 9.7, 13.4, 16.2, 16.6, and 16.8-16.11. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector can be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, cleavage) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of the heterologous PNS SCP protein. Furthermore, different vector/host expression systems can effect processing reactions such as proteolytic cleavages to different extents.

As discussed above, expression of PNS SCP in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis. See, *e.g.*, Ausubel, *infra*; Sambrook, *infra*.

Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) can be introduced into an appropriate host cell by any of a variety of suitable means, *i.e.*, transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene molecule(s) results in the production of at least one PNS SCP. This can take place in the transformed cells as such, or following the induction of these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like).

**Isolation of PNS SCP.** The PNS SCP proteins or fragments of this invention can be obtained by expression from recombinant DNA as described above. Alternatively, a PNS SCP can be purified from biological material. If so desired, the expressed at least one PNS SCP can be isolated and purified in accordance with conventional method steps, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis, or the like. For example, cells expressing at least one PNS SCP in suitable levels can be collected by centrifugation, or with suitable buffers, lysed, and the protein isolated by column chromatography, for example, on DEAE-cellulose, phosphocellulose, polyribocytidylic acid-agarose, hydroxyapatite or by electrophoresis or immunoprecipitation. Alternatively, PNS SCPs



can be isolated by the use of specific antibodies, such as, but not limited to, an PNS SCP or SC antibody. Such antibodies can be obtained by known method steps (*see, e.g. Colligan, infra; Ausubel, infra*).

For purposes of the invention, one method of purification which is illustrative, without being limiting, consists of the following steps. A first step in the purification of a PNS SCP includes extraction of the PNS SCP fraction from a biological sample, such as peripheral nerve tissue or dorsal root ganglia (DRG), in buffers, with or without solubilizing agents such as urea, formic acid, detergent, or thiocyanate. A second step includes subjecting the solubilized material to ion-exchange chromatography on Mono-Q or Mono-S columns (Pharmacia LKB Biotechnology, Inc; Piscataway, NJ). Similarly, the solubilized material can be separated by any other process wherein molecules can be separated according to charge density, charge distribution and molecular size, for example. Elution of the PNS SCP from the ion-exchange resin are monitored by an immunoassay, such as M-IRMA, on each fraction. Immunoactive peaks would be then dialyzed, lyophilized, and subjected to molecular sieve, or gel chromatography. In a third step, molecular sieve or gel chromatography is a type of partition chromatography in which separation is based on molecular size. Dextran, polyacrylamide, and agarose gels are commonly used for this type of separation. One useful gel for the invention is SEPHAROSE 12 (Pharmacia LKB Biotechnology, Inc.). However, other methods, known to those of skill in the art can be used to effectively separate molecules based on size. A fourth step in a purification protocol for a PNS SCP can include analyzing the immunoactive peaks by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a further gel chromatographic purification step, and staining, such as, for example, silver staining. A fifth step in a purification method can include subjecting the PNS SCP obtained after SDS-PAGE to affinity chromatography, or any other procedure based upon affinity between a substance to be isolated and a molecule to which it can specifically bind. For further purification of a PNS SCP, affinity chromatography on SEPHAROSE conjugated to anti-PNS SCP mAbs (specific mAbs generated against substantially pure PNS SCP) can be used. Alternative methods, such as reverse-phase HPLC, or any other method characterized by rapid separation with good peak resolution are useful.

It will be appreciated that other purification steps can be substituted for the preferred method described above. Those of skill in the art will be able to devise alternate purification schemes without undue experimentation.

***An Antibody Having Binding Affinity to a PNS SCP Peptide and a Hybridoma Containing the Antibody.*** In another embodiment, the invention relates to an antibody having binding affinity specifically to a PNS SCP peptide as described above or fragment thereof. Those which bind selectively to PNS SCP would be chosen for use in methods which could include, but should not be limited to, the analysis of altered PNS SCP expression in tissue containing PNS SCP.

The PNS SCP proteins of the invention can be used in a variety of procedures and methods, such as for the generation of antibodies, for use in identifying pharmaceutical compositions, and for studying DNA/protein interaction.

The PNS SCP peptide of the invention can be used to produce antibodies or hybridomas. One skilled in the art will recognize that if an antibody is desired, such a peptide would be generated as described herein and used as an immunogen.

The antibodies of the invention include monoclonal and polyclonal antibodies, as well as fragments of these antibodies. The invention further includes single chain antibodies. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques.

The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, anti-idiotypic (anti-Id) antibodies to antibodies that can be labeled in soluble or bound form, as well as fragments thereof provided by any known technique, such as, but not limited to enzymatic cleavage, peptide synthesis or recombinant techniques. Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen. A monoclonal antibody contains a substantially homogeneous population of antibodies specific to antigens, which population contains substantially similar epitope binding sites. MAbs can be obtained by methods known to those skilled in the art. See, e.g., Kohler and Milstein, *Nature* 256:495-497 (1975); U.S. Patent No. 4,376,110; Ausubel et al., eds., *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1987, 1992); and Harlow and Lane *ANTIBODIES: A LABORATORY MANUAL* Cold Spring Harbor Laboratory (1988); Colligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), the contents of which references are incorporated entirely herein by reference. Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, GILD and any subclass thereof. A hybridoma producing a mAb of the invention can be cultivated *in vitro*, *in situ* or *in vivo*. Production of high titers of mAbs *in vivo* or *in situ* makes this the presently preferred method of production.

Chimeric antibodies are molecules different portions of which are derived from different animal species, such as those having variable region derived from a murine mAb and a human immunoglobulin constant region, which are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine mAbs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric mAbs are used. Chimeric antibodies and methods for their production are known in the art (Cabilly et al., *Proc. Natl. Acad. Sci. USA* 81:3273-3277 (1984); Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Boulianne et al., *Nature* 312:643-646 (1984); Cabilly et al., European Patent Application 125023; Neuberger et al., *Nature* 314:268-270 (1985); Taniguchi et al., European Patent Application 171 496; Morrison et al., European Patent Application 173 494; Neuberger et al., PCT Application WO 86/01533; Kudo et al., European Patent Application 184 187; Morrison et al., European Patent Application 173 494; Sahagan et al., *J. Immunol.* 137:1066-1074 (1986); Robinson et al., International Patent Publication No. PCT/US86/02269; Liu et al., *Proc. Natl. Acad. Sci. USA* 84:3439-3443 (1987); Sun et al., *Proc. Natl. Acad. Sci. USA* 84:214-218 (1987); Better et al., *Science* 240:1041-1043 (1988); and Harlow, *infra*. These references are entirely incorporated herein by reference.

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type (e.g., mouse strain) as the source of the mAb with the mAb to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotype determinants of the immunizing antibody by producing an antibody to these idiotype determinants (the anti-Id antibody). See, for example, U.S. patent No. 4,699,880, which is herein entirely incorporated by reference.

The anti-Id antibody can also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id can be epitopically identical to the original mAb which induced the anti-Id. Thus, by using antibodies to the idiotype determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

Accordingly, mAbs generated against a PNS SCP of the invention can be used to induce anti-Id antibodies in suitable animals, such as BALB/c mice. Spleen cells from such immunized mice are used to produce anti-Id hybridomas secreting anti-Id mAbs. Further, the anti-Id mAbs can be coupled to a carrier such as keyhole limpet hemocyanin (KLH) and used to immunize additional BALB/c mice. Sera from these mice will contain anti-Id antibodies that have the binding properties of the original mAb specific for a PNS SCP specific epitope. The anti-Id mAbs thus have their own idiotypic epitopes, or "idiotopes" structurally similar to the epitope being evaluated.

The term "antibody" is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and F(ab')<sub>2</sub>, which are capable of binding antigen. Fab and F(ab')<sub>2</sub> fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and can have less non-specific tissue binding than an intact antibody (Wahl *et al.*, *J. Nucl. Med.* 24:316-325 (1983)). It will be appreciated that Fab and F(ab')<sub>2</sub> and other fragments of the antibodies useful in the invention can be used for the detection and/or quantitation of a PNS SCP according to the methods disclosed herein for intact antibody molecules. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The term "epitope" is meant to refer to that portion of any molecule capable of being bound by an antibody which can also be recognized by that antibody. Epitopes or "antigenic determinants" usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics.

An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen can have one, or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which can be evoked by other antigens.

**Immunoassays.** Antibodies of the invention, directed against a PNS SCP, can be used to detect or diagnose a PNS SC or a PNS SC-related pathologies. Screening methods are provided by the invention can include, *e.g.*, immunoassays employing radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) methodologies, based on the production of specific antibodies (monoclonal or polyclonal) to a PNS SCP. For these assays, biological samples are obtained by, nerve biopsy, or other peripheral nervous system tissue sampling. For example, in one form of RIA, the substance under test is mixed with diluted antiserum in the presence of radiolabeled antigen. In this method, the concentration of the test substance will be inversely proportional to the amount of labeled antigen bound to the specific antibody and directly related to the amount of free labeled antigen. Other suitable screening methods will be readily apparent to those of skill in the art.

Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques, methods and kits disclosed above with regard to antibodies, to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby *et al.*, "Application of Synthetic Peptides: Antisense Peptides", In: *Synthetic Peptides, A User's Guide*, W.H. Freeman, NY, pp. 289-307 (1992), and Kaspczak *et al.*, *Biochemistry* 28:9230-8 (1989).

One embodiment for carrying out the diagnostic assay of the invention on a biological sample containing a PNS SCP, comprises:

- (a) contacting a detectably labeled PNS SCP-specific antibody with a solid support to effect immobilization of said PNS SCP-specific antibody or a fragment thereof;
- (b) contacting a sample suspected of containing a PNS SCP with said solid support;
- (c) incubating said detectably labeled PNS SCP-specific antibody with said support for a time sufficient to allow the immobilized PNS SCP-specific antibody to bind to the PNS SCP;
- (d) separating the solid phase support from the incubation mixture obtained in step (c); and
- (e) detecting the bound label and thereby detecting and quantifying PNS SCP.

The specific concentrations of detectably labeled antibody and PNS SCP, the temperature and time of incubation, as well as other assay conditions can be varied, depending on various factors including the concentration of a PNS SCP in the sample, the nature of the sample, and the like. The binding activity of a given lot of anti-PNS SCP antibody can be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation. Other such steps as washing, stirring, shaking, filtering and the like can be added to the assays as is customary or necessary for the particular situation.

Detection can be accomplished using any of a variety of assays. For example, by radioactively labeling the PNS SCP-specific antibodies or antibody fragments, it is possible to detect PNS SCP through the use of radioimmune assays. A good description of a radioimmune assay can be found in Colligan, *infra*, and Ausubel, *infra*, entirely incorporated by reference herein. Preferably, the detection of cells which express a PNS SCP can be accomplished by *in vivo* imaging techniques, in which the labeled antibodies (or fragments thereof) are provided to a subject, and the presence of the PNS SCP is detected without the prior removal of any tissue sample. Such *in vivo* detection procedures have the advantage of being less invasive than other detection methods, and are, moreover, capable of detecting the presence of PNS SCP in tissue which cannot be easily removed from the patient, such as brain tissue.

There are many different *in vivo* labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the invention include radioactive isotopes and paramagnetic isotopes. Those of ordinary skill in the art will know of other suitable labels for binding to the antibodies used in the invention, or will be able to ascertain such, using routine experimentation. Furthermore, the binding of these labels to the antibodies can be done using standard techniques common to those of ordinary skill in the art.

For diagnostic *in vivo* imaging, the type of detection instrument available is a major factor in selecting a given radionuclide. The radionuclide chosen must have a type of decay which is detectable for a given type of instrument. In general, any conventional method for visualizing diagnostic imaging can be utilized in accordance with this invention. For example, positron emission tomography (PET), gamma, beta, and magnetic resonance imaging (MRI) detectors can be used to visualize diagnostic imaging.

The antibodies useful in the invention can also be labeled with paramagnetic isotopes for purposes of *in vivo* diagnosis. Elements which are particularly useful, as in Magnetic Resonance Imaging (MRI), include  $^{151}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ , and  $^{59}\text{Fe}$ .

The antibodies (or fragments thereof) useful in the invention are also particularly suited for use *in vitro* immunoassays to detect the presence of a PNS SCP in body tissue, fluids (such as CSF), or cellular extracts. In such immunoassays, the antibodies (or antibody fragments) can be utilized in liquid phase or, preferably, bound to a solid-phase carrier, as described above.

5        *In situ* detection can be accomplished by removing a histological specimen from a patient, and providing the combination of labeled antibodies of the invention to such a specimen. The antibody (or fragment) is preferably provided by applying or by overlaying the labeled antibody (or fragment) to a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of a PNS SCP, but also the distribution of a PNS SCP on the examined tissue. Using the invention, those of ordinary skill will readily perceive that any of a wide variety of  
10        histological methods (such as staining procedures) can be modified in order to achieve such *in situ* detection.

As used herein, an effective amount of a diagnostic reagent (such as an antibody or antibody fragment) is one capable of achieving the desired diagnostic discrimination and will vary depending on such factors as age, condition, sex, the extent of disease of the subject, counter-indications, if any, and other variables to be adjusted by the physician. The amount of such materials which are typically used in a diagnostic test are generally between 0.1 to 5 mg, and  
15        preferably between 0.1 to 0.5 mg.

The assay of the invention is also ideally suited for the preparation of a kit. Such a kit can comprise a carrier means being compartmentalized to receive in close confinement therewith one or more container means such as vials, tubes and the like, each of said container means comprising the separate elements of the immunoassay.

For example, there can be a container means containing a first antibody immobilized on a solid phase support,  
20        and a further container means containing a second detectably labeled antibody in solution. Further container means can contain standard solutions comprising serial dilutions of the PNS SCP to be detected. The standard solutions of a PNS SCP can be used to prepare a standard curve with the concentration of PNS SCP plotted on the abscissa and the detection signal on the ordinate. The results obtained from a sample containing a PNS SCP can be interpolated from such a plot to give the concentration of the PNS SCP.

25        **Diagnostic Screening and Treatment.** It is to be understood that although the following discussion is specifically directed to human patients, the teachings are also applicable to any animal that expresses at least one PNS SC. The diagnostic and screening methods of the invention are especially useful for a patient suspected of being at risk for developing a disease associated with an altered expression level of PNS SCP based on family history, or a patient in which it is desired to diagnose a PNS SCP-related disease.

30        According to the invention, presymptomatic screening of an individual in need of such screening is now possible using DNA encoding the PNS SCP protein of the invention. The screening method of the invention allows a presymptomatic diagnosis, including prenatal diagnosis, of the presence of a missing or aberrant PNS SC gene in individuals, and thus an opinion concerning the likelihood that such individual would develop or has developed a PNS SC-associated disease. This is especially valuable for the identification of carriers of altered or missing PNS SC genes,  
35        for example, from individuals with a family history of a PNS SC-related pathology. Early diagnosis is also desired to maximize appropriate timely intervention.

In one preferred embodiment of the method of screening, a tissue sample would be taken from such individual, and screened for (1) the presence of the "normal" PNS SCP gene; (2) the presence of PNS SCP mRNA and/or (3) the

presence of PNS SCP protein. The normal human gene can be characterized based upon, for example, detection of restriction digestion patterns in "normal" versus the patient's DNA, including RFLP analysis, using DNA probes prepared against the PNS SCP sequence (or a functional fragment thereof) taught in the invention. Similarly, PNS SCP mRNA can be characterized and compared to normal PNS SCP mRNA (a) levels and/or (b) size as found in a human population not at risk of developing PNS SCP-associated disease using similar probes. Lastly, PNS SCP protein can be (a) detected and/or (b) quantitated using a biological assay for PNS SCP activity or using an immunological assay and PNS SCP antibodies. When assaying PNS SCP protein, the immunological assay is preferred for its speed. An (1) aberrant PNS SCP DNA size pattern, and/or (2) aberrant PNS SCP mRNA sizes or levels and/or (3) aberrant PNS SCP protein levels would indicate that the patient is at risk for developing a PNS SCP-associated disease.

The screening and diagnostic methods of the invention do not require that the entire PNS SCP DNA coding sequence be used for the probe. Rather, it is only necessary to use a fragment or length of nucleic acid that is sufficient to detect the presence of the PNS SCP gene in a DNA preparation from a normal or affected individual, the absence of such gene, or an altered physical property of such gene (such as a change in electrophoretic migration pattern).

Prenatal diagnosis can be performed when desired, using any known method to obtain fetal cells, including amniocentesis, chorionic villous sampling (CVS), and fetoscopy. Prenatal chromosome analysis can be used to determine if the portion of the chromosome possessing the normal PNS SCP gene is present in a heterozygous state.

**Overview of PNS SCP Purification and Crystallization Methods.** In general, a PNS SCP as a membrane protein, is purified in soluble form using detergents (e.g., octylglucosides) or other suitable amphiphilic molecules. The resulting PNS SCP is in sufficient purity and concentration for crystallization. The purified PNS SCP is then isolated and assayed for biological activity and for lack of aggregation (which interferes with crystallization). The purified and cleaved PNS SCP preferably runs as a single band under reducing or nonreducing polyacrylamide gel electrophoresis (PAGE) (nonreducing is used to evaluate the presence of cysteine bridges). The purified PNS SCP is preferably crystallized under varying conditions of at least one of the following: pH, buffer type, buffer concentration, salt type, polymer type, polymer concentration, other precipitating ligands and concentration of purified and cleaved PNS SCP by known methods. See, e.g., Michel, *Trends in Biochem. Sci.* 8:56-59 (1983); Deisenhofer et al. *J. Mol. Biol.* 180:385-398 (1984); Weiss et al. *FEBS Lett.* 267:268-272 (1990). Blundell, et al. *Protein Crystallography* Academic Press, London (1976); Oxender et al. eds., *Protein Engineering* Liss, New York (1986); McPherson; *The Preparation and Analysis of Protein Crystals* Wiley, N.Y. (1982); or the methods provided in a commercial kit, such as CRYSTAL SCREEN (Hampton Research, Riverside, CA). The crystallized protein is also tested for at least one SC activity and differently sized and shaped crystals are further tested for suitability in X-ray diffraction. Generally, larger crystals provide better crystallography than smaller crystals, and thicker crystals provide better crystallography than thinner crystals. See, e.g., Blundell, *infra*; Oxender, *infra*; McPherson, *infra*; Wyckoff et al. eds., *Diffraction Methods for Biological Macromolecules*, Vols. 114-115: *Methods in Enzymology*, Orlando, FL Academic Press (1985).

**Protein Crystallization Methods.** The hanging drop method is preferably used to crystallize a purified soluble, PNS SCP protein. See, e.g., Taylor et al., *J. Mol. Biol.* 226:1287-1290 (1992); Takimoto et al. (1992), *infra*; CRYSTAL SCREEN, Hampton Research.. A mixture of the protein and precipitant can include the following: • pH (e.g., 4-10); • buffer type (e.g., tromethamine (TRIZMA), sodium azide, phosphate, sodium, or cacodylate acetates, imidazole, Tris HCl, sodium hepes); • buffer concentration (e.g., 0.1-100 mM); • salt type (e.g., sodium azide, calcium chloride,

sodium citrate, magnesium chloride, ammonium acetate, ammonium sulfate, potassium phosphate, magnesium acetate, zinc acetate; calcium acetate); • polymer type and concentration: (e.g., polyethylene glycol (PEG) 1-50%, type 6000-10,000); • other precipitating ligands (salts: potassium, sodium, tartrate, ammonium sulfate, sodium acetate, lithium sulfate, sodium formate, sodium citrate, magnesium formate, sodium phosphate, potassium phosphate; organics: 2-propanol; non-volatile: 2-methyl-2,4-pentanediol); and • concentration of purified PNS SCP (e.g., 0.1-100 mg/ml, with added amphiphilic molecules (detergents such as octylglucosides)). See, e.g., CRYSTAL SCREEN, Hampton Research.

The above mixtures are used and screened by varying at least one of pH, buffer type; buffer concentration, precipitating salt type or concentration, PEG type, PEG concentration, and cleaved protein concentration. Crystals ranging in size from 0.1-1.5 mm are formed in 1-14 days. These crystals diffract X-rays to at least 10 Å resolution, such as 1.5-10.0 Å, or any range of value therein, such as 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5 or 3, with 3.5 Å or less being preferred for the highest resolution. In addition to diffraction patterns having this highest resolution, lower resolution, such as 25-3.5 Å can further be used.

**Protein Crystals.** Crystals appear after 1-14 days and continue to grow on subsequent days. Some of the crystals are removed, washed, and assayed for biological activity, which activity is preferred for using in further characterizations. Other washed crystals are preferably run on a stained gel and those that migrate in the same position as the purified cleaved PNS SCP are preferably used. From two to one hundred crystals are observed in one drop and crystal forms can occur, such as, but not limited to, bipyramidal, rhomboid, and cubic. Initial X-ray analyses are expected to indicate that such crystals diffract at moderately high to high resolution. When fewer crystals are produced in a drop, they can be much larger size, e.g., 0.2-1.5 mm.

**PNS SCP X-ray Crystallography Methods.** The crystals so produced for a PNS SCP are X-ray analyzed using a suitable X-ray source. A suitable number of diffraction patterns are obtained. Crystals are preferably stable for at least 10 hrs in the X-ray beam. Frozen crystals (e.g., -220 to -50°C) are optionally used for longer X-ray exposures (e.g., 4-72 hrs), the crystals being relatively more stable to the X-rays in the frozen state. To collect the maximum number of useful reflections, multiple frames are optionally collected as the crystal is rotated in the X-ray beam, e.g., for 12-96 hrs. Larger crystals (>0.2 mm) are preferred, to increase the resolution of the X-ray diffraction. Crystals are preferably analyzed using a synchrotron high energy X-ray source. Using frozen crystals, X-ray diffraction data is collected on crystals that diffract to a resolution of 10-1.5 Å, with lower resolutions also useful, such as 25-10 Å, sufficient to solve the three-dimensional structure of a PNS SCP in considerable detail, as presented herein.

**Computer Related Embodiments.** An amino acid sequence of a PNS SCP and/or x-ray diffraction data, useful for computer molecular modeling of a PNS SCP or a portion thereof, can be "provided" in a variety of mediums to facilitate use thereof. As used herein, provided refers to a manufacture, which contains a PNS SCP amino acid sequence and/or x-ray diffraction data of the present invention, e.g., the amino sequence provided in Figures 1, 8, 10 or 11, a representative fragment thereof, or an amino acid sequence having at least 80-100% overall identity to a 5-2005 amino acid fragment of an amino acid sequence of Figures 11A-D or a variant thereof. Such a method provides the amino acid sequence and/or x-ray diffraction data in a form which allows a skilled artisan to analyze and molecular model the three dimension structure of a PNS SCP or subdomain thereof.

In one application of this embodiment, PNS SCP, or at least one subdomain thereof, amino acid sequence and/or x-ray diffraction data of the present invention is recorded on computer readable medium. As used herein, "computer readable medium" refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as optical discs or CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a n amino acid sequence and/or x-ray diffraction data of the present invention.

As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently know methods for recording information on computer readable medium to generate manufactures comprising an amino acid sequence and/or x-ray diffraction data information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon an amino acid sequence and/or x-ray diffraction data of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the sequence and x-ray data information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of dataprocessor structuring formats (e.g. text file or database) in order to obtain computer readable medium having recorded thereon the information of the present invention.

By providing the PNS SCP sequence and/or x-ray diffraction data on computer readable medium, a skilled artisan can routinely access the sequence and x-ray diffraction data to model a PNS SCP, a subdomain thereof, or a ligand thereof. Computer algorithms are publicly and commercially available which allow a skilled artisan to access this data provided in a computer readable medium and analyze it for molecular modeling and/or RDD.

The present invention further provides systems, particularly computer-based systems, which contain the sequence and/or diffraction data described herein. Such systems are designed to do molecular modeling and RDD for a PNS SCP or at least one subdomain thereof.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the sequence and/or x-ray diffraction data of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate which of the currently available computer-based system are suitable for use in the present invention.

As stated above, the computer-based systems of the present invention comprise a data storage means having stored therein a PNS SCP or fragment sequence and/or x-ray diffraction data of the present invention and the necessary hardware means and software means for supporting and implementing an analysis means. As used herein, "data storage means" refers to memory which can store sequence or x-ray diffraction data of the present invention, or a memory



access means which can access manufactures having recorded thereon the sequence or x-ray data of the present invention.

As used herein, "search means" or "analysis means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence or x-ray data stored within the data storage means. Search means are used to identify fragments or regions of a PNS SCP which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting computer analyses that can be adapted for use in the present computer-based systems.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration or electron density map which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzymic active sites, structural subdomains, epitopes, functional domains and signal sequences. A variety of structural formats for the input and output means can be used to input and output the information in the computer-based systems of the present invention.

A variety of comparing means can be used to compare a target sequence or target motif with the data storage means to identify structural motifs or electron density maps. A skilled artisan can readily recognize that any one of the publicly available computer modeling programs can be used as the search means for the computer-based systems of the present invention.

One application of this embodiment is provided in Figure 12. Figure 12 provides a block diagram of a computer system 102 that can be used to implement the present invention. The computer system 102 includes a processor 106 connected to a bus 104. Also connected to the bus 104 are a main memory 108 (preferably implemented as random access memory, RAM) and a variety of secondary storage memory 110, such as a hard drive 112 and a removable storage medium 114. The removable medium storage device 114 may represent, for example, a floppy disk drive, a CD-ROM drive, a magnetic tape drive, etc. A removable storage medium 116 (such as a floppy disk, a compact disk, a magnetic tape, etc.) containing control logic and/or data recorded therein may be inserted into the removable medium storage medium 114. The computer system 102 includes appropriate software for reading the control logic and/or the data from the removable medium storage device 114 once inserted in the removable medium storage device 114. A monitor 120 can be used as connected to the bus 104 to visualize the structure determination data.

Amino acid, encoding nucleotide or other sequence and/or x-ray diffraction data of the present invention may be stored in a well known manner in the main memory 108, any of the secondary storage devices 110, and/or a removable storage device 116. Software for accessing and processing the amino acid sequence and/or x-ray diffraction data (such as search tools, comparing tools, etc.) reside in main memory 108 during execution.

**Three Dimensional Structure Determination.** One or more computer modeling steps and/or computer algorithms are used to provide a molecular 3-D model of a cleaved PNS SCP, using amino acid sequence data from Figures 1, 8, 10 or 11 (or variants thereof) and/or x-ray diffraction data. If only the amino acid sequence is used, for three-dimensional structure determination then a suitable modeling program can be used, e.g., LINUS (Rose *et al.* *Proteins: Structure, Function and Genetics* (June, 1995) and references cited herein. It is preferred that the PNS SCP model has no or Ala-substituted (for surface) residues in disallowed regions of the Ramachandran plot, and gives a positive 3D-1D profile (Luthy *et al.*, *Nature* 356:83-85 (1992)), suggesting that all the residues are in acceptable environments (Kraulis (1991), *infra*). Alternatively, the disallowed regions can be corrected by the use of suitable algorithms, such as the RAVE program described herein. Phase determination is optionally used for solving the three-dimensional structure of a cleaved PNS SCP. This structure can then be used for RDD of modulators of PNS SCP neuraminidase, endothelin cathepsin A or other biological activity, e.g., which is relevant to a PNS SCP related pathology.

**Density Modification and Map Interpretation.** Electron density maps can be calculated using such programs as those from the CCP4 computing package (SERC (UK) Collaborative Computing Project 4, Daresbury Laboratory, UK, 1979). Cycles of two-fold averaging can further be used, such as with the program RAVE (Kleywegt & Jones, Bailey *et al.*, eds., *First Map to Final Model*, SERC Daresbury Laboratory, UK, pp 59-66 (1994)) and gradual model expansion. For map visualization and model building a program such as "O" (Jones (1991), *infra*) can be used.

**Refinement and Model Validation.** Rigid body and positional refinement can be carried out using a program such as X-PLOR (Brünger (1992), *infra*), e.g., with the stereochemical parameters of Engh and Huber (*Acta Cryst.* A47:392-400 (1991)). If the model at this stage in the averaged maps still misses residues (e.g., at least 5-10 per subunit), the some or all of the missing residues can be incorporated in the model during additional cycles of positional refinement and model building. The refinement procedure can start using data from lower resolution (e.g., 25-10 Å to 10-3.0 Å and then gradually extended to include data from 12-6 Å to 3.0-1.5 Å).  $\beta$ -values for individual atoms can be refined once data between 2.9 and 1.5 Å has been added. Subsequently waters can be gradually added. A program such as ARP (Lamzin and Wilson, *Acta Cryst.* D49:129-147 (1993)) can be used to add crystallographic waters and as a tool to check for bad areas in the model. Programs such as PROCHECK (Lackowski *et al.*, *J. Appl. Cryst.* 26:283-291 (1993)), WHATIF (Vriend, *J. Mol. Graph.* 8:52-56 (1990)) and PROFILE 3D (Luthy *et al.*, *Nature* 356:83-85 (1992)), as well as the geometrical analysis generated by X-PLOR can be used to check the structure for errors. For the final refinement cycle, 20-5% of the weakest data can be rejected using a  $IF_{obs}/\sigma$  cutoff and anisotropic scaling between  $F_{obs}$  and  $F_{calc}$  applied after careful assessment of the quality and completeness of the data

**Structure Analysis.** A program such as DSSP can be used to assign the secondary structure elements (Kabsch and Sander (1983), *infra*). A program such as SUPPOS (from the BIOMOL crystallographic computing package) can be used to for some or all of the least-squares superpositions of various models and parts of models. Solvent accessible surfaces and electrostatic potentials can be calculated using such programs as GRASP (Nicholls *et al.* (1991), *infra*).

**Structure Determination.** The structure of a PNS SCP can thus be solved with the molecular replacement procedure such as by using X-PLOR (Brünger (1992), *infra*). A partial search model for the monomer can be constructed using a related protein, such as wheat serine carboxypeptidase structure (Liao *et al.* (1992), *infra*). The rotation and translation function can be used to yield two or more orientations and positions for two subunits to form

a physiological dimer as determined based on their interactions. Cyclical two-fold density averaging can also be done using the RAVE program and model expansion can also be used to add missing residues for each monomer, resulting in a model with 95-99.9% of the total number residues. The model can be refined in a program such as X-PLOR (Brünger (1992), *supra*), to a suitable crystallographic  $R_{\text{factor}}$ . The model data is then saved on computer readable medium for use in further analysis, such as rational drug design.

**Rational Design of Drugs that Interact with the PNS SCP.** The determination of the three dimensional structure of a cleaved PNS SCP, as described herein, provides a basis for the design of new and specific ligands for the diagnosis and/or treatment of at least one PNS SCP-related pathology. Several approaches can be taken for the use of the crystal structure of a PNS SCP in the rational design of ligands of this protein. A computer-assisted, manual examination of the active site structure is optionally done. The use of software such as GRID (Goodford, *J. Med. Chem.* 28:849-857 (1985)) a program that determines probable interaction sites between probes with various functional group characteristics and the enzyme surface — is used to analyze the active site to determine structures of inhibiting compounds. The program calculations, with suitable inhibiting groups on molecules (e.g., protonated primary amines) as the probe, are used to identify potential hotspots around accessible positions at suitable energy contour levels. Suitable ligands, as inhibiting or stimulating modulating compounds or compositions, are then tested for modulating activities of at least one PNS SCP.

A diagnostic or therapeutic PNS SCP modulating ligand of the present invention can be, but is not limited to, at least one selected from a nucleic acid, a compound, a protein, an element, a lipid, an antibody, a saccharide, an isotope, a carbohydrate, an imaging agent, a lipoprotein, a glycoprotein, an enzyme, a detectable probe, and antibody or fragment thereof, or any combination thereof, which can be detectably labeled as for labeling antibodies. Such labels include, but are not limited to, enzymatic labels, radioisotope or radioactive compounds or elements, fluorescent compounds or metals, chemiluminescent compounds and bioluminescent compounds. Alternatively, any other known diagnostic or therapeutic agent can be used in a method of the invention.

After preliminary experiments are done to determine the  $K_m$  of the substrate with each enzyme activity of a PNS SCP, the time-dependent nature of modulation of ligand  $K_i$  values are determined, (e.g., by the method of Henderson (*Biochem. J.* 127:321-333 (1972))). For example, the substrate (or blank where appropriate) and enzyme are pre-incubated in buffer. Reactions are initiated by the addition of substrate. Aliquots are removed over a suitable time course and each quenched by addition into the aliquots of suitable quenching solution (e.g., sodium hydroxide in aqueous ethanol). The concentration of product is determined, e.g., fluorometrically, using a spectrometer. Plots of fluorescence against time can be close to linear over the assay period, and are used to obtain values for the initial velocity in the presence ( $V_i$ ) or absence ( $V_o$ ) of ligand. Error is present in both axes in a Henderson plot, making it inappropriate for standard regression analysis (Leatherbarrow, *Trends Biochem. Sci.* 15:455-458 (1990)). Therefore,  $K_i$  values is obtained from the data by fitting to a modified version of the Henderson equation for competitive inhibition:

$$Qr^2 + (E_i - Q - I)r - E_i = 0$$

where (using the notation of Henderson (*Biochem. J.* 127:321-333 (1972)):

$$Q = K_i \left( \frac{A_i + K_e}{K_e} \right) \quad \text{and} \quad r = \frac{V_e}{V_i}$$

This equation is solved for the positive root with the constraint that

$$Q = K_i((A_i + K_e) / K_e)$$

using PROCNLIN from SAS (SAS Institute Inc., Cary, North Carolina, USA) which performs nonlinear regression using least-square techniques. The iterative method used is optionally the multivariate secant method, similar to the Gauss-Newton method, except that the derivatives in the Taylor series are estimated from the histogram of iterations rather than supplied analytically. A suitable convergence criterion is optionally used, e.g., where there is a change in loss function of less than  $10^{-4}$ .

Once modulating ligands are found and isolated or synthesized, crystallographic studies of the compounds complexed to a PNS SCP are performed. As a non-limiting example, PNS SCP crystals are soaked for 2 days in 0.01-100 mM ligand and X-ray diffraction data are collected on an area detector and/or an image plate detector (e.g., a Mar image plate detector) using a rotating anode X-ray source. Data are collected to as high a resolution as possible, e.g., 1.5-3.5 Å, and merged with an *R*-factor on suitable intensities. An atomic model of the inhibitor is built into the difference Fourier map ( $F_{\text{inhibitor complex}} - F_{\text{native}}$ ). The model can be refined to a solution in a cycle of simulated annealing (Brünger (1987), *infra*) involving 10-500 cycles of energy refinement, 100-10,000 1-FS steps of room temperature dynamics and/or 10-500 more cycles of energy refinement. Harmonic restraints are also used for the atom refinement, except for atoms within a 10-15 Å radius of the inhibitor. An *R*-factor is selected for the model for both the r.m.s. deviations from the ideal bond lengths, as well as for the angles, respectively. Direct measurements of enzyme inhibition provide further confirmation that the modeled ligands are modulators of at least one biological activity of a PNS SC.

Ligands of a PNS SCP, based on the crystal structure of this enzyme, are thus also provided by the present invention. Demonstration of clinically useful levels, e.g., *in vivo* activity is also important. In evaluating PNS SCP inhibitors for biological activity in animal models (e.g., rat, mouse, rabbit) using various oral and parenteral routes of administration are evaluated. Using this approach, it is expected that modulation of a PNS SCP occurs in suitable animal models, using the ligands discovered by molecular modeling and x-ray crystallography.

**Diagnostic and/or Therapeutic Agents.** A diagnostic or therapeutic PNS SCP modulating agent or ligand of the present invention can be, but is not limited to, at least one selected from a nucleic acid, a compound, a protein, an element, a lipid, an antibody, a saccharide, an isotope, a carbohydrate, an imaging agent, a lipoprotein, a glycoprotein, an enzyme, a detectable probe, and antibody or fragment thereof, or any combination thereof, which can be detectably labeled as for labeling antibodies, as described herein. Such labels include, but are not limited to, enzymatic labels, radioisotope or radioactive compounds or elements, fluorescent compounds or metals, chemiluminescent compounds and bioluminescent compounds. Alternatively, any other known diagnostic or therapeutic agent can be used in a method of the invention.

A therapeutic agent used in the invention can have a therapeutic effect on the target cell as a cell or neuron of the peripheral nervous system, the effect selected from, but not limited to: correcting a defective gene or protein, a drug action, a toxic effect, a growth stimulating effect, a growth inhibiting effect, a metabolic effect, a catabolic effect, an anabolic effect, a neurohumoral effect, a cell differentiation stimulatory effect, a cell differentiation inhibitory effect, a neuromodulatory effect, a pluripotent stem cell stimulating effect, and any other known therapeutic effects that modulates at least one SC in a cell of the peripheral nervous system can be provided by a therapeutic agent delivered to a target cell via pharmaceutical administration or via a delivery vector according to the invention.

A therapeutic nucleic acid as a therapeutic agent can have, but is not limited to, at least one of the following therapeutic effects on a target cell: inhibiting transcription of a DNA sequence; inhibiting translation of an RNA sequence; inhibiting reverse transcription of an RNA or DNA sequence; inhibiting a post-translational modification of a protein; inducing transcription of a DNA sequence; inducing translation of an RNA sequence; inducing reverse transcription of an RNA or DNA sequence; inducing a post-translational modification of a protein; transcription of the nucleic acid as an RNA; translation of the nucleic acid as a protein or enzyme; and incorporating the nucleic acid into a chromosome of a target cell for constitutive or transient expression of the therapeutic nucleic acid.

Therapeutic effects of therapeutic nucleic acids can include, but are not limited to: turning off a defective gene or processing the expression thereof, such as antisense RNA or DNA; inhibiting viral replication or synthesis; gene therapy as expressing a heterologous nucleic acid encoding a therapeutic protein or correcting a defective protein; modifying a defective or underexpression of an RNA such as an hnRNA, an mRNA, a tRNA, or an rRNA; encoding a drug or prodrug, or an enzyme that generates a compound as a drug or prodrug in pathological or normal cells expressing the chimeric receptor; and any other known therapeutic effects.

A therapeutic nucleic acid of the invention which encodes, or provides the therapeutic effect any known toxin, prodrug or gene drug for delivery to pathogenic nervous cells can also include genes under the control of a tissue specific transcriptional regulatory sequence (TRSs) specific for pathogenic SC containing cells. Such TRSs would further limit the expression of the therapeutic agent in the target cell, according to known methods.

Non-limiting examples of such PNS SCP modulating agents or ligands of the present invention and methods thereof include methyl/halophenyl-substituted piperazine compounds, such as lidoflazine (see, e.g., Merck Index Monograph 5311 and U.S. patent No. 3,267,104, both entirely incorporated herein by reference). Such compounds were tested and found to inhibit sodium channel activity of at least one PNS SCP of the present invention in cell lines expressing at least one PNS SCP, such as PC12, PK1-4 and other isolated or recombinant cells expressing at least one PNS SCP of the present invention. Accordingly, the present invention provides PNS SCP modulating agents or ligands as methyl/halophenyl-substituted piperazines. The substitutions can include alkyl- and/or halophenyl-substituted piperazines.

**Pharmaceutical/Diagnostic Administration.** Using PNS SCP modulating compounds or compositions (including antagonists and agonists as described above) the present invention further provides a method for modulating the activity of the PNS SCP protein in a cell. In general, agents (antagonists or agonists) which have been identified to inhibit or enhance the activity of PNS SCP can be formulated so that the agent can be contacted with a cell expressing a PNS SCP protein *in vivo*. The contacting of such a cell with such an agent results in the *in vivo* modulation of the

activity of the PNS SCP proteins. So long as a formulation barrier or toxicity barrier does not exist, agents identified in the assays described above will be effective for *in vivo* use.

In another embodiment, the invention relates to a method of administering PNS SCP or a PNS SCP modulating compound or composition (including PNS SCP antagonists and agonists) to an animal (preferably, a mammal (specifically, a human)) in an amount sufficient to effect an altered level of PNS SCP in the animal. The administered PNS SC or PNS SCP modulating compound or composition could specifically effect PNS SCP associated functions. Further, since PNS SCP is expressed in peripheral nervous system tissue, administration of PNS SC or PNS SCP modulating compound or composition could be used to alter PNS SCP levels in the peripheral nervous system.

PNS SCP antagonists can be used to treat pain due to trauma or pathology involving the central or peripheral nervous system, or pathologies related to the abnormally high levels of expression of at least one naturally occurring nervous system specific (NS) sodium channel (SC), where a PNS SCP antagonist also inhibits at least one NS SC, or where the pain is mediated to some extent by PN SC. Such pathologies, include, but are not limited to; inflammatory diseases, neuropathies (e.g., diabetic neuropathy), dystrophies (e.g., reflex sympathetic dystrophy, post-herpetic neuralgia); trauma (tissue damage by any cause); focal pain by any cause.

Inflammatory diseases can include, but are not limited to, chronic inflammatory pathologies and vascular inflammatory pathologies. Chronic inflammatory pathologies include, but are not limited to sarcoidosis, chronic inflammatory bowel disease, ulcerative colitis, and Crohn's pathology and vascular inflammatory pathologies, such as, but not limited to, disseminated intravascular coagulation, atherosclerosis, and Kawasaki's pathology.

PNS SCP agonists can be used to treat pathologies involving the central or peripheral nervous system, or pathologies related to the abnormally low levels of expression of at least one naturally occurring nervous system specific (NS) sodium channel (SC), where a PNS SCP agonist also enhances or stimulates at least one NS SC. Such pathologies, include, but are not limited to, neurodegenerative diseases, diseases of the gastrointestinal tract due to dysfunction of the enteric nervous system (e.g., colitis, ileitis, inflammatory bowel syndrome); diseases of the cardiovascular system (e.g., hypertension and congestive heart failure); diseases of the genitourinary tract involving sympathetic and parasympathetic innervation (e.g., benign prostrate hyperplasia, impotence); diseases of the neuromuscular system (e.g., muscular dystrophy, multiple sclerosis, epilepsy).

Neurodegenerative diseases can include, but are not limited to, *demyelinating diseases*, such as multiple sclerosis and acute transverse myelitis; *hyperkinetic movement disorders*, such as Huntington's Chorea and senile chorea; *hypokinetic movement disorders*, such as Parkinson's disease; *progressive supranuclear palsy*; *spinocerebellar degenerations*, such as spinal ataxia, Friedreich's ataxia; multiple systems degenerations (Mencel, Dejerine-Thomas, Shi-Drager, and Machado-Joseph); and systemic disorders (Refsum's disease, abetalipoproteinemia, ataxia, telangiectasia, and mitochondrial multi-system disorder); *demyelinating core disorders*, such as multiple sclerosis, acute transverse myelitis; *disorders of the motor unit*, such as neurogenic muscular atrophies (anterior horn cell degeneration, such as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy); or any subset thereof.

Pharmaceutical/diagnostic administration of diagnostic/pharmaceutical compound or composition of the invention, for a PNS SC related pathology can be administered by any means that achieve its intended purpose, for example, to treat or prevent a cancer or precancerous condition.

The term "protection", as in "protection from infection or disease", as used herein, encompasses "prevention," "suppression" or "treatment." "Prevention" involves administration of a Pharmaceutical composition *prior to the induction* of the disease. "Suppression" involves administration of the composition *prior to the clinical appearance* of the disease. "Treatment" involves administration of the protective composition *after the appearance* of the disease. It will be understood that in human and veterinary medicine, it is not always possible to distinguish between "preventing" and "suppressing" since the ultimate inductive event or events can be unknown, latent, or the patient is not ascertained until well after the occurrence of the event or events. Therefore, it is common to use the term "prophylaxis" as distinct from "treatment" to encompass both "preventing" and "suppressing" as defined herein. The term "protection," as used herein, is meant to include "prophylaxis." See, e.g., Berker, *infra*, Goodman, *infra*, Avery, *infra* and Katzung, *infra*, which are entirely incorporated herein by reference, including all references cited therein. The "protection" provided need not be absolute, i.e., the disease need not be totally prevented or eradicated, provided that there is a statistically significant improvement relative to a control population. Protection can be limited to mitigating the severity or rapidity of onset of symptoms of the disease.

At least one PNS SC modulating compound or composition of the invention can be administered by any means that achieve the intended purpose, using a pharmaceutical composition as previously described.

For example, administration can be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, intracranial, transdermal, or buccal routes. Alternatively, or concurrently, administration can be by the oral route. Parenteral administration can be by bolus injection or by gradual perfusion over time.

An additional mode of using of a diagnostic/pharmaceutical compound or composition of the invention is by topical application. A diagnostic/pharmaceutical compound or composition of the invention can be incorporated into topically applied vehicles such as salves or ointments.

For topical applications, it is preferred to administer an effective amount of a diagnostic/pharmaceutical compound or composition according to the invention to target area, e.g., skin surfaces, mucous membranes, and the like, which are adjacent to peripheral neurons which are to be treated. This amount will generally range from about 0.0001 mg to about 1 g of a PNS SC modulating compound per application, depending upon the area to be treated, whether the use is diagnostic, prophylactic or therapeutic, the severity of the symptoms, and the nature of the topical vehicle employed. A preferred topical preparation is an ointment, wherein about 0.001 to about 50 mg of active ingredient is used per cc of ointment base.

A typical regimen for treatment or prophylaxis comprises administration of an effective amount over a period of one or several days, up to and including between one week and about six months.

It is understood that the dosage of a diagnostic/pharmaceutical compound or composition of the invention administered *in vivo* or *in vitro* will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the diagnostic/ pharmaceutical effect desired. The ranges of effective doses provided herein are not intended to be limiting and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one skilled in the relevant arts. See, e.g., Berkow *et al.*, eds., *The Merck Manual*, 16th edition, Merck and Co., Rahway, N.J., 1992; Goodman *et al.*, eds., *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 8th edition,

Pergamon Press, Inc., Elmsford, N.Y., (1990); *Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics*, 3rd edition, ADIS Press, LTD., Williams and Wilkins, Baltimore, MD. (1987); Ebadi, *Pharmacology*, Little, Brown and Co., Boston, (1985); Osol et al., eds., *Remington's Pharmaceutical Sciences*, 18th edition, Mack Publishing Co., Easton, PA (1990); Katzung, *Basic and Clinical Pharmacology*, Appleton and Lange, Norwalk, CT (1992), which references are entirely incorporated herein by reference.

The total dose required for each treatment can be administered by multiple doses or in a single dose. The diagnostic/pharmaceutical compound or composition can be administered alone or in conjunction with other diagnostics and/or pharmaceuticals directed to the pathology, or directed to other symptoms of the pathology.

Effective amounts of a diagnostic/pharmaceutical compound or composition of the invention are from about 0.1 µg to about 100 mg/kg body weight, administered at intervals of 4-72 hours, for a period of 2 hours to 1 year, and/or any range or value therein, such as 0.0001-1.0, 1-10, 10-50 and 50-100, 0.0001-0.001, 0.001-0.01, 0.01-0.1, 0.1-1.0, 1.0-10, 5-10, 10-20, 20-50 and 50-100 mg/kg, at intervals of 1-4, 4-10, 10-16, 16-24, 24-36, 36-48, 48-72 hours, for a period of 1-14, 14-28, or 30-44 days, or 1-24 weeks, or any range or value therein.

The recipients of administration of compounds and/or compositions of the invention can be any vertebrate animal, such as mammals, birds, bony fish, frogs and toads. Among mammals, the preferred recipients are mammals of the Orders Primata (including humans, apes and monkeys), Arteriodactyla (including horses, goats, cows, sheep, pigs), Rodenta (including mice, rats, rabbits, and hamsters), and Carnivora (including cats, and dogs). Among birds, the preferred recipients are turkeys, chickens and other members of the same order. The most preferred recipients are humans.

**Gene Therapy.** A delivery vector of the present invention can be, but is not limited to, a viral vector, a liposome, an anti-PNS SCP or anti-SC antibody, or a SC ligand, one or more of which delivery vectors is associated with a diagnostic or therapeutic agent.

The delivery vector can comprise any diagnostic or therapeutic agent which has a therapeutic or diagnostic effect on the target cell. The target cell specificity of the delivery vector is thus provided by use of a target cell specific delivery vector.

The delivery vector can also be a recombinant viral vector comprising at least one binding domain selected from the group consisting of an antibody or fragment, a chimeric binding site antibody or fragment, a target cell or specific ligand, a receptor which binds a target cell ligand, an anti-idiotypic antibody, a liposome or other component which is specific for the target cell. A PNS SCP can be already associated with the target cell, or the delivery vector can bind the target cell via a ligand to a target cell receptor or vice versa.

Thus, the therapeutic or diagnostic agent, such as a therapeutic or diagnostic nucleic acid, protein, drug, compound composition and the like, is delivered preferentially to the target cell, e.g., where the nucleic acid is preferably incorporated into the chromosome of the target cell, to the partial or complete exclusion of non-target cells.

The invention is thus intended to provide delivery vectors, containing one or more therapeutic and/or diagnostic agents, including vectors suitable for gene therapy.

In a method of treating a PNS SCP-associated disease in a patient in need of such treatment, functional PNS SCP DNA can be provided to the PNS cells of such patient in a manner and amount that permits the expression of the PNS SCP protein provided by such gene, for a time and in a quantity sufficient to treat such patient, such as a suitable



delivery vector. Many vector systems are known in the art to provide such delivery to human patients in need of a gene or protein missing from the cell. For example, retrovirus systems can be used, especially modified retrovirus systems and especially herpes simplex virus systems. Such methods are provided for, in, for example, the teachings of Breakefield, et al., *The New Biologist* 3:203-218 (1991); Huang, Q. et al., *Experimental Neurology* 115:303-316 (1992), WO93/03743 and WO90/09441. Delivery of a DNA sequence encoding a functional PNS SCP protein will effectively replace the missing or mutated PNS SCP gene of the invention.

In another embodiment of this invention, the PNS SCP modulating compound or composition is expressed as a recombinant gene in a cell, so that the cells can be transplanted into a mammal, preferably a human in need of gene therapy. To provide gene therapy to an individual, a genetic sequence which encodes for all or part of the PNS SCP modulating compound or composition is added into a vector and introduced into a host cell. Examples of diseases that can be suitable for gene therapy include, but are not limited to, neurodegenerative diseases or disorders, Alzheimer's, schizophrenia, epilepsy, neoplasms and cancer. Examples of vectors that can be used in gene therapy include, but are not limited to, defective retroviral, adenoviral, or other viral vectors (Mulligan, R.C., *Science* 260:926-932 (1993)). See Anderson, *Gene Therapy*, 246 J. Amer. Med. Assn. 2737 (1980); Friedmann, *Progress toward human gene therapy*, 244 *Science* 1275 (1989); Anderson, 256 *Science* 808 (1992); human gene therapy protocols published in *Human Gene Therapy*, Mary Ann Liebert Publishers, N.Y. (1990-1994); Bank et al., 565 *Ann. N.Y. Acad. Sci.* 37 (1989); LTR-Vectors (U.S. Patent No. 4,405,712); Ausubel, *infra*, §§ 9.10-9.17; Jon A. Wolff, ed., *Gene Therapeutics: methods and applications of direct gene transfer*, Birkhäuser, Boston (1994).

The means by which the vector carrying the gene can be introduced into the cell include but is not limited to, microinjection, electroporation, transduction, or transfection using DEAE-Dextran, lipofection, calcium phosphate or other procedures known to one skilled in the art (Sambrook *infra*; Ausubel, *infra*).

Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives can also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like. See, generally, Osol et al., eds. *Remington's Pharmaceutical Science*, 16th Ed., (1980).

In another embodiment, the invention relates to a pharmaceutical composition comprising PNS SC or PNS SCP modulating compound or composition in an amount sufficient to alter PNS SCP associated activity, and a pharmaceutically acceptable diluent, carrier, or excipient. Appropriate concentrations and dosage unit sizes can be readily determined by one skilled in the art (See, e.g., Osol et al. ed., *Remington's Pharmaceutical Sciences*, 16th Ed., Mack, Easton PA (1980) and WO 91/19008).

Included as well in the invention are pharmaceutical compositions comprising an effective amount of at least one PNS SCP antisense oligonucleotide, in combination with a pharmaceutically acceptable carrier. Such antisense oligos include, but are not limited to, at least one nucleotide sequence of 12-500 bases in length which is complementary

to a DNA sequence of SEQ ID NO:1, or a DNA sequence encoding at least 4 amino acids of SEQ ID NO:2 or Figure 11A-11E.

Alternatively, the PNS SCP nucleic acid can be combined with a lipophilic carrier such as any one of a number of sterols including cholesterol, cholate and deoxycholic acid. A preferred sterol is cholesterol.

5 The PNS SCP gene therapy nucleic acids and the pharmaceutical compositions of the invention can be administered by any means that achieve their intended purpose. For example, administration can be by parenteral, subcutaneous, intravenous, intramuscular, intra-peritoneal, or transdermal routes. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

10 Compositions within the scope of this invention include all compositions wherein the PNS SCP antisense oligonucleotide is contained in an amount effective to achieve enhanced expression of at least one PNS SCP in a peripheral nervous system neuron or ganglion. While individual needs vary, determination of optimal ranges of effective amounts of each component is with the skill of the art. Typically, the PNS SCP nucleic acid can be administered to mammals, e.g. humans, at a dose of 0.005 to 1 mg/kg/day, or an equivalent amount of the pharmaceuti-  
15 cally acceptable salt thereof, per day of the body weight of the mammal being treated.

Suitable formulations for parenteral administration include aqueous solutions of the PNS SCP nucleic acid in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions can be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions can  
20 contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension can also contain stabilizers.

Alternatively, at least one PNS SCP can be coded by DNA constructs which are administered in the form of virions, which are preferably incapable of replicating *in vivo* (see, for example, Taylor, WO 92/06693). For example, such DNA constructs can be administered using herpes-based viruses (Gage *et al.*, U.S. Patent No. 5,082,670).  
25 Alternatively, PNS SCP antisense RNA sequences, PNS SCP ribozymes, and PNS SCP EGS can be coded by RNA constructs which are administered in the form of virions, such as recombinant, replication deficient retroviruses or adenoviruses. The preparation of retroviral vectors is well known in the art (see, for example, Brown *et al.*, "Retroviral Vectors," in *DNA Cloning: A Practical Approach*, Volume 3, IRL Press, Washington, D.C. (1987)).

30 Specificity for gene expression in the peripheral nervous system can be conferred by using appropriate cell-specific regulatory sequences, such as cell-specific enhancers and promoters. Since protein phosphorylation is critical for neuronal regulation (Kennedy, "Second Messengers and Neuronal Function," in *An Introduction to Molecular Neurobiology*, Hall, Ed., Sinauer Associates, Inc. (1992)), protein kinase promoter sequences can be used to achieve sufficient levels of PNS SCP gene expression.

Thus, gene therapy can be used to alleviate sodium channel related pathology by inhibiting the inappropriate  
35 expression of a particular form of PNS SC. Moreover, gene therapy can be used to alleviate such pathologies by providing the appropriate expression level of a particular form of PNS SCP. In this case, particular PNS SCP nucleic acid sequences can be coded by DNA or RNA constructs which are administered in the form of viruses, as described above.

Having now generally described the invention, the same will be more readily understood through reference to the following Examples which are provided by way of illustration, and are not intended to be limiting of the invention, unless specified.

### Example 1:

#### Cloning and Sequencing of a PNS SC Encoding Nucleic Acid

##### Materials and Methods

**Cell Culture.** PC12 cells and PKI-4 PC12 subclones were grown as previously described (Mandel *et al.*, 1988). NGF (2.5 S subunit, kindly supplied by Dr. S. Halegoua, SUNY at Stony Brook), was added to the culture medium at final concentration of 110 ng/ml. The PKI-4 PC12 subclone which expresses the cAMP-dependent kinase inhibitor protein (PKI) was also provided by Dr. S. Halegoua (see D'Arcangelo *et al.*, *J. Cell Biol.* 122:915-921 (1993)).

**PCR Amplification.** Total cellular RNA was isolated, according to the method of Cathala *et al.* *DNA* 2:329-335 (1983), from a PC12 subclone (PKI-4) which expresses high levels of the cAMP-dependent protein kinase inhibitor protein. Two µg of total RNA prepared time NGF-treated PKI-4 cells was used to synthesize first strand cDNA using random hexamer primers for the reverse transcriptase reaction. The cDNA then served as template for the PCR amplification, using a pair of degenerate oligonucleotide primers that specified a 400 base pair region within repeat domain III of the sodium channel  $\alpha$  subunit gene. The 5' primer (designated YJ1: GCGAAGCTTTC)TATTTT(TC)I(GATC)IAT(ATC)ATGGG (SEQ ID NO:3), underline indicates a *Hind*III restriction site), corresponded to amino acids FWLIFSIM at positions 1347-1354 in the type II sodium channel gene.

The 3' primer (designated YO1C: GCA G G A T C C (AG)TT(AG)AAA(AG)TT(AG)TC(AGT)AT(AGCT)AC(AGCT)CC (SEQ ID NO:5), underline indicates a *Bam*HI restriction site) corresponded to amino acids GVIIDNFN at positions 1470-1447 in the type II gene. The amplification reaction mixture consisted of 5% of the cDNA, 1 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 0.5 µM each primer, *Taq* polymerase (Perkin-Elmer) in a buffer consisting of 0.1 M KCl, 0.1 M TRIS HCl (pH 8.3) and gelatin (1 mg/ml). The reaction was performed in a Perkin-Elmer thermocycler as follows: 5 cycles of denaturation (94°C, 1 min.), annealing (37°C, 1 min.), and extension (72°C, 1 min) followed by 25 cycles of denaturation (94°C, 1 min.), annealing (50°C, 1 min.) and extension (72°C, 1 min.). The PCR products were excised from a low melt agarose gel (SEAPLAQUE GTG, FMC BIOPRODUCTS) and subcloned into a Bluescript II SK plasmid vector previously restricted with *Hind*III and *Bam*HI. The clones were screened for cDNA inserts by miniprep (Sambrook *et al.*, *infra*) and sequenced in both directions by dideoxy chain termination (Sequenase 2.0 kit, UNITED STATES BIOCHEMICAL). Sequence data was compiled and analyzed using GENWORKS software (INTELLIGENETICS, INC., Mountain View, CA).

**cDNA Library Construction and Screening.** Poly(A)<sup>+</sup> mRNA from the PKI-4 PC12 subclone was purified (mRNA purification kit, PHARMACIA) and used to construct a random- and oligo (dT)-primed Lambda ZAP II cDNA library (STRATAGENE CORP., La Jolla, CA). The library consisted of 5.6 X 10<sup>6</sup> independent clones prior to amplification. Screening of approximately 4 X 10<sup>6</sup> recombinants using the cloned PCR product pPC12-1 labeled by random primers (PHARMACIA kit) resulted in isolation of 5 cDNAs ranging in size from 1-3 kb. Sequence analysis and comparison to published sequences established that the two of the cDNAs together encoded 3033 bp of the novel sodium channel  $\alpha$  subunit, PN1.

**Northern blot analysis and ribonuclease protection assays.** Total cellular RNA was isolated from adult Sprague-Dawley rat brain, spinal cord, superior cervical ganglion, dorsal root ganglion, skeletal muscle, cardiac muscle, and adrenal gland using the standard method of Chirgwin, *Biochemistry* 18:5294-5299 (1979). RNA was electrophoresed and transferred to nylon membrane as previously described (Cooperman *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:8721 (1987)) (DURALON-UV; STRATAGENE CORP.). RNA blots were cross-linked to the nylon using Stratalinker UV crosslinker (STRATAGENE CORP.) and hybridized to <sup>32</sup>P-UTP-labeled antisense RNA probes generated from the following linearized templates: pPC12-1, pRB211 (Cooperman, *infra*, 1987), p1B15 (cyclophilin; Danielson *et al.*, *DNA* 7:261-267 (1988)), and rat brain type I, which contains 51 bp of intron, 5' untranslated sequence and 267 bp of coding sequence of the type I sodium channel. RNA probes were transcribed with either T3 (pPC12-1), T7, (pNach1), or SP6 (pRB211, p1B15) RNA polymerase according to the manufacturer's instructions (PROMEGA CORP, Madison, WI). The blots were washed once in 2 x SSC, 0.1% NaDodSO<sub>4</sub>, for 15 min. at 68°C, followed by two washes in 0.2 x SSC, 0.1% NaDodSO<sub>4</sub>, for 15 min. at 68°C. Autoradiography with preflashed XAR-5 film (EASTMAN KODAK CO., Rochester, NY) was used for quantitation of mRNA by densitometry.

Ribonuclease protection assays were performed by use of a kit (RPA II, AMBION INC., Austin, TX). Total RNA was hybridized with 10<sup>6</sup> cpm of antisense RNA probe generated from pPC12-1. To control for differences in the amount of total RNA between samples, we included an antisense RNA probe for  $\beta$  actin, transcribed from pTRI- $\beta$ -actin (AMBION, INC.).

**In situ hybridization.** Tissue preparation and hybridization were performed using a modification of the procedure described by Yokouchi *et al.*, *Develop.* 113:431-444 (1991). SCG and DRG were dissected from adult Sprague-Dawley rats and fixed in 4% paraformaldehyde (in 0.1 M PBS) for 2-6 hrs. at 4°C. The tissue was then rinsed = 5 min. in 0.1 M PBS (pH 7.3), cryoprotected in 30% sucrose (in 0.1 M PBS) for 2 hrs. at 4°C and embedded in O.C.T. (TISSUE-TEK). Cryostat sections (14  $\mu$ M) were collected on SUPERFROST/Plus slides (FISHER SCIENTIFIC), dried = 2 hrs. at room temp., and then stored at -80°C.

Immediately before prehybridization, sections were brought to room temp. and rehydrated in 0.1 M PBS (pH 7.3) containing 0.3% Triton X-100 for 5 min. Sections were then treated with 0.2 N HCl for 20 min., washed in 0.1 M PBS for 5 min., and digested with proteinase K (5  $\mu$ g/ml in 0.1 M PBS) for 40 min. at 37°C. Sections were then postfixed with 4% paraformaldehyde (in 0.1 M PBS), rinsed with 0.1 M PBS containing 0.1 M glycine for 15 min., and equilibrated in 50% formamide, 2 x SSC for 1 hr. (room temp.).

Sections were hybridized with antisense digoxigenin-labeled RNA probes transcribed from pPC12-1 or pNach2 (Cooperman *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:8721 (1987)) according to the manufacturer's instructions for RNA labeling with digoxigenin-UTP (BOEHRINGER MANNHEIM). Unlabeled probes were synthesized by replacing digoxigenin-UTP with rUTP. Each section was covered with = 100  $\mu$ l of hybridization solution containing 20 mM TRIS HCl (pH 8.0), 2.5 mM EDTA, 50% formamide, 0.3 M NaCl, 1x Denhardt's, 10% dextran sulfate, 1 mg/ml tRNA, and probe at a concentration of 0.7  $\mu$ g/ml. Sections were then covered with PARAFILM coverslips and incubated in a humid chamber overnight at 45°C. After hybridization, sections were washed in 50% formamide, 2 x SSC at 45°C for 1 hr., followed by RNase digestion in 0.5M NaCl, 10 mM TRIS HCl (pH 8.0), and 20  $\mu$ g/ml RNase A (BOEHRINGER MANNHEIM). Sections were subsequently washed at 45°C in 50% formamide, 2 x SSC for 1 hr., and 50% formamide, 1 x SSC for 1 hr.

Immunological detection was performed using a kit (GENIUS 3 KIT, BOEHRINGER MANNHEIM), according to the manufacturer's instructions. In most experiments, the sections were incubated in the color solution for  $\approx$  3-5 hrs. at room temp. Sections were then coverslipped with AQUA-MOUNT (Lerner Laboratories) and stored in the dark.

**Densitometry.** Levels of sodium channel mRNA were determined by densitometric analysis of the autoradiograms using Bio Image software (Millipore Corp., Ann Arbor, Michigan). Levels of RNA were normalized to the quantitated levels of cyclophilin mRNA.

## Results

**Isolation of a cDNA expressed preferentially in peripheral nerve.** D'Arcangelo *et al.*, *J. Cell Biol.* 122:915-921 (1993) showed previously that NGF treatment of PC12 cells increase the level of an  $\approx$  11 kb sodium channel gene transcript which did not hybridize to probes specific for any of the known sodium channel genes. A transcript identical in size was also detected in mRNA from adult rat sympathetic and sensory ganglia, but not in mRNA from brain. These results suggested that the transcript encoded a new member of the sodium channel gene family (termed Peripheral Nerve type 1 (PN1)).

To confirm the identity of the PN1 gene, cDNAs from an NGF-treated PC12 subclone which preferentially expresses PN1 mRNA (PKI-4 cells) D'Arcangelo *et al.* were amplified by the polymerase chain reaction (PCR), using a pair of degenerate oligonucleotide primers that specify a 400 base pair (bp) region of the sodium channel  $\alpha$  subunit gene (see Methods, Figure 1). Both primers specified putative membrane-spanning regions within repeat domain III, which are highly conserved among voltage-gated sodium channels. The amplified regions between the primers include the strictly-conserved pore-lining residues, as well as residues which are divergent among the different mammalian  $\alpha$  subunits. Sequence analysis of the PCR products revealed a cDNA, pPC12-1, which encoded a portion of a novel putative sodium channel  $\alpha$  subunit (Figure 1). Additional cDNAs were further isolated which encapsulated the entire PN1 coding region.

To determine whether pPC12-1 encode part of the PN1 gene, the cDNA was used to generate antisense RNA probes for Northern blot analysis of mRNA from control and NGF-treated PC12 cells (Figure 2B). For comparison, a duplicate blot (Figure 2A) was hybridized with an antisense probe pRB211, which encode a highly-conserved region of the sodium channel  $\alpha$  subunit (Cooperman *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:8721 (1987)) and which cross-hybridizes with the PN1 transcript, and that, as shown by D'Arcangelo *et al.*, *J. Cell Biol.* 122:915-921 (1993), levels of the detected transcript should increase rapidly and transiently following NGF treatment (maximal  $\approx$  5 hrs). Comparison of Figures 2A and 2B shows that pPC12-1 fulfilled both of these criteria. Also, consistent with D'Arcangelo *et al.*, *J. Cell Biol.* 122:915-921 (1993), we found that NGF induction of the transcript detected by pPC12-1 is independent of cAMP-dependent protein kinase activity.

To isolate additional cDNAs encoding PN1, a random- and oligo (dT)-primed Lambda ZAP II cDNA library (STRATAGENE,  $5.6 \times 10^6$  independent clones) was prepared from poly(A) + mRNA isolated from the same PC12 subclone from which pPC12-1 was isolated. Screening  $4 \times 10^6$  recombinants with a probe generated from pPC12-1 resulted in isolation of 2 additional, overlapping cDNAs which are joined to give a 3033 bp cDNA (Figure 7). Additional cDNAs were further isolated which encapsulated the entire PN1 coding region.

**Analysis of the deduced primary structure of PN1.** As shown in Figure 8, the deduced primary structure of PN1 encodes repeat domain II of the sodium channel  $\alpha$  subunit gene. Comparison with the type II sodium channel shows that the PN1 sequence contains all of the structural motifs characteristic of voltage-gated sodium channels, including six putative transmembrane domains (IIIS1-IIIS6). The S4 domain, thought to serve as the voltage sensor, exhibits the highly-conserved pattern of a positively-charged residue (lysine or arginine) at every third position. Furthermore, the putative pore-lining segments (IIIS1-IIIS2) contain residues shown to be involved in sodium-selective permeation (Heinemann *et al.*, *Nature* 356:441-443 (1992)) as well as TTX affinity (Terlaue *et al.*, *FEBS Lett.* 293:93-96 (1991)).

In addition to such highly-conserved structural features, the sodium channel  $\alpha$  subunit undergoes several characteristic post-translational modifications. All sodium channels sequenced to date exhibit a distinctive pattern of asparagine-linked (N-linked) glycosylation sites, which are found almost exclusively in the extracellular loops joining the S5 and S6 transmembrane helices. The N-linked glycosylation sites of PN1 are in good agreement with this pattern; three potential extracellular glycosylation sites are located between IIIS5 and IIIS6. Two of the sites are also found in the types I, II and III sodium channels.

The  $\alpha$  subunit is phosphorylated by protein kinase C (PKC), and deduced PN1 sequence contains the highly-conserved consensus PKC phosphorylation site at serine<sup>1204</sup> (Figure 1). This residue is located in the cytoplasmic loop joining domains III and IV that has been implicated in channel inactivation, and mutational analysis has shown that this serine is required for PKC modulation of channel inactivation (West *et al.*, 1991).

The entire DNA (Figure 9A-D) and amino acid (Figure 10) sequences were determined. The rat PN1 amino acid sequence was compared with new human sequences (Figure 11A-E) presented in Example 2.

In sum, the deduced primary structure of PN1 contains all of the hallmark structural and functional domains characteristics a  $\alpha$  subunit the voltage-gated sodium channel.

**The PN1 gene is expressed preferentially in the PNS.** To determine whether the PN1 gene was expressed preferentially in the PNS, total RNA was isolated from adult rat brain, spinal cord, SCG, DRG, skeletal muscle, and cardiac muscle and subjected to Northern blot analysis. Blots were hybridized with the PN1-specific antisense probe generated from pPC12-1. As shown in Figure 3A, we found high levels of hybridization to an  $\approx$  11 kb transcript in both SCG and DRG. Much lower, but detectable levels hybridization were seen to transcripts in both spinal cord and brain. No detectable hybridization was observed to mRNA from skeletal muscle, cardiac muscle, or liver.

Ribonuclease (RNase) protection analyses were also prepared. Total RNA was isolated from the same tissues used in Northern blot analysis, as well as adrenal gland, and hybridized to PN1-specific antisense probe (pPC12-1). mRNA from SCG, DRG, brain, spinal cord, and adrenal gland protected a 343 bp fragment of the PN1 probe (Figure 4B). The non-protected bases represent oligonucleotide primer and plasmid sequences. The PN1 probe was not protected by mRNA from either skeletal muscle or cardiac muscle.

To determine the relative amounts of PN1 mRNA in the various tissues, autoradiographs from three separate RNase protection experiments were analyzed by densitometry. To control for small differences in the amount of total RNA between samples, we included a probe for a  $\beta$  actin. PN1 mRNA levels in both SCG and DRG are approximately 40-fold greater than in spinal cord, adrenal gland and brain.

*The PN1 gene is expressed in sympathetic and sensory neurons.* To determine whether the PN1 gene is expressed in neurons of peripheral ganglia, *in situ* hybridization was used to examine the cellular distribution of PN1 mRNA in adult rat SCG and DRG. Cryostat sections were hybridized with a PN1-specific digoxigenin-labeled RNA probe (pPC12-1), which was visualized using an anti-digoxigenin antibody conjugated to alkaline phosphatase. As shown in Figure 4A, B the PN1 antisense probe labeled most neuronal cell bodies in both SCG and DRG. To confirm that the hybridization signal was due to binding of the probe specifically to PN mRNA, we performed two different negative controls: (1) Sections were hybridized with the digoxigenin-labeled probe in the presence of a 100-fold excess of unlabeled PN1 antisense probe. (2) Previous experiments have shown that SCG and DRG contain extremely low levels of type II sodium channel mRNA (Beckh, S., *FEBS Lett.* 262:317-322 (1990)). Therefore, we also hybridized sections with a type II-specific antisense probe. As shown, in Figure 4C-F, both of these control experiments greatly reduced the hybridization signal. Also, consistent with the results of Northern blot and RNase protection analyses, we found that hybridization of the labeled PN1 probe to sections of adult rat cerebral cortex yielded no detectable staining.

Although the PN1 probe stained most neuronal cell bodies in both SCG and DRG, we found that cell-to-cell variability in PN1 mRNA levels differed between the two ganglia. SCG neurons were fairly homogeneous, in that the intensity of reaction product was relatively constant between different cells. DRG neurons, however, were quite heterogeneous in that the staining intensity varied considerably from cell to cell. For example, in Figure 4B, arrows indicate two DRG neurons of approximately the same diameter which differ markedly in staining intensity.

Finally, we found that the PN2 probe did not stain non-neuronal cells such as satellite cells and Schwann cells. However, it is possible that these cells contain very low levels of PN1 mRNA which are not detectable by this method.

*SCG neurons also express the type I sodium channel gene.* Earlier Northern blot analysis has shown that mRNA from SCG contains two distinct sodium channel gene transcripts. As we have demonstrated, the larger, 11 kb transcript encodes the PN1 sodium channel. The smaller transcript, however, has not yet been identified. We hypothesized that this smaller transcript encoded the type I sodium channel, because moderate levels of type I mRNA have been found in other PNS tissues (Beckh, S., *FEBS Lett.* 262:317-322 (1990)). To test this hypothesis, Northern blots of SCG mRNA isolated from adult rats were hybridized with an antisense probe specific for the type I sodium channel gene (pNchl, see Methods above). As shown in Figure 5, the type I-specific probe hybridized specifically to the smaller transcript. Furthermore, we have found that SCG mRNA protects the type I probe in an RNase protection assay.

*The putative PN1 $\alpha$  subunit and type I $\alpha$  subunit genes are differentially regulated during development.* Several studies have shown that the types I, II and III sodium channel genes are differentially regulated during development in both the central and peripheral nervous systems. To determine whether the PN1 and type I genes are also independently regulated during development, we measured their relative mRNA levels in SCG isolated from rats of different postnatal ages. To visualize both transcripts simultaneously, Northern blots were hybridized with the conserved sodium channel gene probe pRB211. As shown in Figure 6A, in SCG removed on postnatal day 7 (P7), the levels of PN1 and type I mRNA are approximately equal. However, by P14, their relative abundance has shifted such that level of PN1 mRNA exceeds that of type I by  $\approx 2$ -fold. This increase in ratio of PN1 to type I mRNA levels continues for at least the next four postnatal weeks. By P42, PN1 is the predominant sodium channel gene transcript, with levels of PN1 mRNA several-fold greater than that of type I.

To quantitate the development changes in mRNA levels, autoradiographs from three separate experiments were analyzed by densitometry. To control for differences in the amount of total RNA between lanes, blots were subsequently hybridizing blots with a probe for the internal control cyclophilin. As shown in Figure 6B, in which percent maximum mRNA is plotted versus postnatal age, the shift in relative abundance of the two transcripts in largely due to a developmental decrease in level of type I sodium channel mRNA. From P7 to P42, the level of type I mRNA decreases by approximately 80%.

### **Example 2: Drug Screening for PN-1 Antagonists**

The ability of a PNS SCP-ligand (e.g., antagonists and agonists) to inhibit or enhance the activity of a PNS SCP is evaluated with cells expressing at least one PNS SCP. An assay for PNS SCP activity in such cells is used to determine the functionality of the PNS SCP protein in the presence of at least one agent which can act as antagonist or agonist, and thus, agents that interfere or enhance the activity of PNS SCP are identified. Two or more cell lines (each expressing a different PNS SCP) are used, as well as optionally using one or more cell lines expressing a CNS specific sodium channel as a control.

These agents are selected and screened (1) at random; (2) by a rational selection; and or (3) by design using for example, computer modeling techniques.

There are numerous variations of assays which can be used by a skilled artisan without the need for undue experimentation in order to isolate, modulating agents or ligands of a PNS SCP. Agent determination methods include Computer Assisted Molecular Design (CAMD), PNS SCP-agent binding, sophisticated chemical synthesis and testing, targeted screening, peptide combinatorial library technology, antisense technology and/or biological assays, according to known methods. See, e.g., Rapaka et al., eds., *Medications Development: Drug Discovery, Databases, and Computer-Aided Drug Design*, NIDA Research Monograph 134, NIH Publication No. 93-3638, U.S. Dept. of Health and Human Services, Rockville, MD (1993); Langone, *Methods in Enzymology, Volume 203, Molecular Design and Modeling: Concepts and Applications, Part B, Antibodies and Antigens, Nucleic Acids, Polysaccharides and Drugs*, Section III, pp 587-702, Academic Press, New York (1991).

Alternatively, cell expression libraries, or other cells are used to that have been selected or genetically engineered to express and display a PNS SCP via the use of the PNS SCP nucleic acids of the invention are preferred in such methods, as host cell lines may be chosen which are devoid of related receptors. Rapaka, *infra*, (1993), at pages 58-65.

A PNS SCP agent in the context of the present invention refers to any chemical or biological molecule that associates with a PNS SCP *in vitro*, *in situ* or *in vivo*, and can be, but is not limited to, synthetic, recombinant or naturally derived chemical compounds and compositions, e.g., organic compounds, nucleic acids, peptides, carbohydrates, vitamin derivatives, hormones, neurotransmitters, viruses or receptor binding domains thereof, opsins, rhodopsins, nucleosides, nucleotides, coagulation cascade factors, odorants or pheromones, toxins, growth factors, platelet activating factors, neuroactive peptides, neurohumors, or any biologically active compound, such as drugs or naturally occurring compounds.

The agents are selected and screened at random or rationally selected or designed using computer modeling techniques. For random screening, potential agents are selected and assayed for their ability to bind to the PNS SCP,



or a fragment thereof. Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of at least one specific PNS SCP (e.g., as presented in Figure 11). For example, one skilled in the art can readily adapt currently available procedures to generate agents capable of binding to a specific peptide sequence in order to generate rationally designed compounds, such as chemical compounds, nucleic acids or peptides. See, e.g., Rapaka, *infra*, (1993); Hurby *et al.*, "Application of Synthetic Peptides: Antisense Peptides," in *Synthetic Peptides: A User's Guide*, W.H. Freeman, New York (1992), pp. 289-307; and Kaspczak *et al.*, *Biochemistry* 28:9230-2938 (1989).

A method of screening for an agent that modulates the activity of at least one PNS SCP comprising:

(a) incubating at least one cell line expressing at least one PNS SCP with an agent to be tested; and

(b) assaying the at least one cell for the activity of the at least one PNS SCP protein by measuring the agents effect on PNS SCP binding or PNS SCP activity preferably the or assay distinguishes the agent's effect on alternative PNS SCP and determines that the agent has little or no effect on CNS sodium channels, or has relatively less effect on CNS sodium channels..

Any cell can be used in the above assay so long as it expresses a functional form of PNS SCP protein and the PNS SCP activity can be measured. The preferred expression cells are eukaryotic cells or organisms. Such cells can be modified to contain DNA sequences encoding the PNS SCP protein using routine procedures known in the art. Alternatively, one skilled in the art can introduce mRNA encoding the PNS SCP protein directly into the cell.

In an alternative embodiment stem cell populations for either neuronal or glial cells can be genetically engineered to express a functional PNS SCP ion channel. Such cells expressing the PNS SCP ion channel, can be transplanted to the diseased or injured region of the mammal's neurological system (*Neural Transplantation. A Practical Approach*, Donnet & Djorklund, eds., Oxford University Press, New York, NY (1992)). In another embodiment, embryonic tissue or fetal neurons can be genetically engineered to express functional PNS SCP ion channel and transplanted to the diseased or injured region of the mammal's limbic system. The feasibility of transplanting fetal dopamine neurons into Parkinsonian patients has been demonstrated. (Lindvall *et al.*, *Archives of Neurology* 46:615-631 (1989)).

At least two types of approaches are currently used to express voltage-dependent sodium channel clones in order to generate functional channel proteins. In one approach, mRNA encoding the cloned cDNA is expressed in *Xenopus* oocytes. The sodium channel cDNA is cloned into a bacterial expression vector such as the pGEM recombinant plasmid (Meltan, *et al.*, 1984). Transcription of the cloned cDNA is carried out using an RNA polymerase such as SP6 polymerase or T7 polymerase with a capping analog such as M<sup>3</sup>G(5')ppp(5')G. The resulting RNA (e.g., about 50 nl, corresponding to 2-5 ng) is injected into stage V and stage VI oocytes isolated from *Xenopus*, and incubated for 3-5 days at 19°C. Oocytes are tested for sodium channel expression with a two-microelectrode voltage clamp (Trimmer *et al.*, *Neuron* 3:33-49 1989).

In an alternative approach, cDNAs encoding a voltage-dependent sodium channel is cloned into any one of a number of mammalian expression vectors, and transfected into mammalian cells which do not express endogenous voltage-dependent sodium channels (such as fibroblast cell lines). Transfected clones are selected expressing the cloned, transfected cDNA. Sodium channel expression is measured with a whole cell voltage clamp technique using a patch electrode (D'Arcangelo *et al.*, *J. Cell. Biol.* 122:915-921 (1993)).

*Sources of PNS SCPs and Cell Lines Useful for Drug Screening.* Any cell line expressing (Naturally, by induction or due to recombinant expression of a PNS SCP) can be used for drug screening. As a non-limiting example, PC12 cells express both PN1 and Type II sodium channels. A126-1B2 cells are mutants deficient in Protein Kinase A (PKA) activity and which express PN1, but are now discovered to not express Type II sodium channels. PKI-4 is a PC12 cell line transfected with a cDNA encoding a peptide inhibitor of PKA. Each of these cell lines can be used as one source of a PNS SCP of the present invention, or as a cell line itself to use in drug screening. Treatment of PC12 cells with NGF reduces both a PNS SCP (PN1) and type II sodium channels, while NGF induces only PN1 in A126-1B2 cells. PKI-4 cells express a PNS SCP (PN1) without NGF treatment. (D'Arcangelo *et al.*, *J. Cell Biol.* 122:915-921 (1993)).

Additionally or alternatively, heterologous expression systems can also be used in which cell lines (such as Chinese Hamster Ovary cells (CHO)) are stably transfected with a cDNA encoding PN-1. Method steps for transfecting and stably expressing cDNA to form heterologous cell lines, are well known in the art. An advantage of using transfected cells is that clones are obtained that express very high levels of a PNS SCP, such as PN-1.

To screen for PNS SCP modulators, as antagonists or agonists, drugs are examined for their ability to:

- (a) inhibit or enhance the binding of radioligands to a PNS SCP (labeled ligand binding reaction), and/or
- (b) to inhibit or enhance ion flux through the channel of the PNS SCP in a cell line that expresses a PNS SCP.

Labeled ligand binding neurotoxins can be used to characterize PNS sodium channels. For example previous studies have identified at least six distinct neurotoxin binding sites on previously characterized non-PNS sodium channels (reviewed in Lombert *et al.*, *FEB* 219(2):355-359 (1987)). Many of these sites are thought to be allosterically coupled to one another (*for review, see Strichartz et al.*, *Ann. Rev. Neurosci.* 10:237-267 (1987), and references cited therein). In other words, binding of a drug or toxin to a particular neurotoxin site can be sensitive to drug binding at not only that site, but other sites on the channel as well. This is advantageous for a drug screening program in that for a given labeled ligand, the likelihood of identifying agents that preferentially bind to a PNS SCP is increased.

The techniques described herein for measuring labeled ligand binding to a PNS SCP of the invention in intact cells (*e.g.*, PC12 PKI or PNS SCP expressing heterologous cell lines) in suspension are similar to those described previously for radioligand binding to other sodium channels in brain synaptosomal preparations (*see, e.g.*, Catterall *et al.*, *J. Biol. Chem.* 256(17):8922-8927 (1981)). However, it is well recognized by those skilled in the art that these techniques are routinely modified for the use of substrate-attached cells or broken cell preparations, based on the teaching and guidance presented herein.

A126-1B2, PC12, PKI-4 or other cells expressing a PNS SCP cells are grown using standard techniques, and optionally treated with NGF for 1-2 days to induce PN-1 expression. Cells are harvested and tested for ion flux activity with alternative potential agents.

For both radioligands, binding reactions are conducted *e.g.*, at 37°C, then stopped. Samples are quickly filtered with vacuum washed with ice-cold buffer, and bound radioactivity determined by scintillation counting.

Ion Flux directly tests the ability of a potential PNS SCP agent to inhibit or enhance the activity of a PNS SCP function, by their ability to inhibit or enhance the influx of ion tracers through a PNS SCP.

Most previous sodium channel studies have employed  $^{22}\text{Na}$  as a tracer (for example, see Catterall *et al.*, *J. Biol. Chem.* 256(17):8922-8927 (1981)). However, the high toxicity of  $^{22}\text{Na}$  can be a disadvantage for its use in high-throughput drug screening. A less toxic alternative is ( $^{14}\text{C}$ ) guanidinium ion, influx of which has been shown to be a reliable indicator of sodium channel opening (Reith, *Europ. J. Pharmacol.* 188:33-41 (1990)). Accordingly, routine methods can be used to screen compounds for modulating PNS SCP ion channel activity, e.g., ( $^{14}\text{C}$ ) guanidinium ion flux using intact cells expressing at least one PNS SCP. Additionally these methods are well known to be easily modified for use with  $^{22}\text{Na}$ . Similarly, these known method steps could be modified for use with substrate-attached cells or vesicles prepared from broken cells, according to known method steps.

For a guanidinium flux assay the methods for  $^{22}\text{Na}$  are modified from those of Reith (*Europ. J. Pharmacol.* 188:33-41 (1990) for brain synaptosomes), e.g., as described in Example 2 below. Aliquots of a cell suspension containing heterologous cells expressing at least one PNS SCP are incubated for 10 minutes at 37°C in the presence of channel openers (typically, 100  $\mu\text{M}$  veratridine) and test drugs in a total volume of 100  $\mu\text{M}$  (0.20-0.25 mg protein). Ion flux is initiated by the addition of HEPES/TRIS solution also containing 4mM guanidine HCl (final) and 1000 dpm/nmol ( $^{14}\text{C}$ ) guanidine. The reaction is continued for 30 seconds and is stopped by the addition of ice-cold incubation buffer, followed by rapid filtration under vacuum over Whatman GF/C filter. The filters are washed rapidly with ice-cold incubation buffer and radioactivity determined by scintillation counting. Nonspecific uptake is determined in parallel by the inclusion of 1 mM tetrodotoxin during both preincubation and uptake.

Using the guanidinium flux assay several methyl/halophenyl substituted compounds, such as lidoflazine (see, e.g., Merck Index Monograph 5311 and U.S. patent No. 3,267,104, both entirely incorporated herein by reference), were tested and found to inhibit sodium channel activity of at least one PNS SCP of the present invention in cell lines expressing at least one PNS SCP, with a  $\text{pIC}_{50}$  of 6.51 for lidoflazine on PK1-4 cells. Accordingly, the present invention provides PNS SCP modulating agents as methyl/halophenyl-substituted piperazines.

### Example 3: Identification of Human PNS SCP Sequence from a Human Peripheral Nervous System cDNA Library

Similar to the procedures provided in Example 1, a human peripheral nervous system cDNA library (as a human DRG library) was used for polymerase chain reaction (PCR) amplification. The PCR used a 5' primer corresponding to DNA encoding amino acids 604-611 of SEQ ID NO:2, and a corresponding 3' primer encoding amino acids 723-731 of SEQ ID NO:2.

The PCR reaction mixture consisted of 5% of the cDNA, 1 mM  $\text{MgCl}_2$ , 0.2 mM dNTPs, 0.5 mM, each primer, *Taq* polymerase (Perkin-Elmer) in a buffer consisting of 0.1 M KCl, 0.1 M TRIS HCl (pH 8.3) and gelatin (1 mg/ml). The reaction was performed in a Perkin-Elmer thermocycler as follows: five cycles of denaturations (94°C, 1 min.), annealing (37°C, 1 min.), and extension (72°C, 1 min.), followed by 25 cycles of denaturation (94°C, 1 min.), annealing (50°C, 1 min.), and extension (72°C, 1 min.).

The resulting PCR products provided a human amplified cDNA which encoded amino acids 646-658 of SEQ ID NO:2, as presented in Figure 11A-E.

**Example 4:**  
**Cloning and Sequencing of Human PN-1 Sequence from Human Dorsal Root Ganglion cDNA Library**

As in Examples 1 and 3 above, additional PCR primers corresponding to SEQ ID NO:1 are used to isolate clones from the human DRG cDNA library which encompass the entire coding region of one or more human PNS SCPs of the present invention. A 5' primer includes the sequence 5'TTTGTGCCCCACAGACCCAG3' (SEQ ID NO: 13) and a 3' primer includes the sequence 5'ACACAAATCTTGATCTGGAATTGCT3' (SEQ ID NO: 14) or 5'CAACCTCAGACAGAGAG CAATGA 3' (SEQ ID NO: 15), which are used for nested PCR. According to Examples 1 and 3 above, PCR is performed to obtain cDNAs encoding a human PNS SCP.

Additional PCR is performed by "walking" 5' or 3' of the sequence corresponding to the above PCR product. In this way cDNAs encompassing the entire coding region of one or more human PNS SCPs are provided.

The resulting additional cDNA clones or PCR products, encoding the entire human PNS SCP, are subcloned into a plasmid vector previously restricted with suitable restriction sites. The clones are screened for cDNA inserts by miniprep (Sambrook *et al.*, *infra*) and sequenced in both directions by dideoxy chain termination (Sequenase 2.0 kit, United States Biochemical). Sequence data is compiled and analyzed using GeneWorks software (IntelliGenetics, Inc., Mountain View, CA). The expected alternative amino acid sequences for a human PN1 sequence or presented in Figure 11 A-D and as SEQ ID NOS:7, 11 and 12, where Xaa represents 0, 1, 2 or 3 amino acids.

Transcripts of the size of the resulting human PNS SCP are then confirmed to be present in human PNS mRNA or cDNA (encoding a 1970-1990 amino acid sequence of Figure 11A-E). However, as in Example 1, such transcripts are not expected to be detected in mRNA from brain. This expected result confirms new human members of the sodium channel gene family (termed Human Peripheral Nerve type 1 (HUMPNI1A (deposited as ATCC No. \_\_\_\_\_) and HUMPNI1B (Deposited as ATCC No. \_\_\_\_\_) of Figure 11A-E, where X is 0, 1, 2 or 3 of the same or different amino acid).

Complete DNA and amino acid sequences of novel human PN1s are then confirmed and are expected to contain all of the structural and functional domain characteristics of an  $\alpha$  subunit of a mammalian voltage-gated sodium channel.

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. The foregoing description of the specific embodiments was not to be taken as limiting the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

*What Is Claimed Is:*

1. An isolated nucleic acid molecule coding for, or complementary to, a peptide comprising an amino acid sequence corresponding to at least one peripheral nervous system specific (PNS) sodium channel peptide (SCP), wherein said SCP has sodium channel (SC) biological activity.
- 5 2. An isolated nucleic acid molecule according to claim 1, wherein the molecule hybridizes under stringent conditions to at least one portion of at least 30 contiguous nucleotides of SEQ ID NO:1 or complementary sequence thereof, which portion encodes at least one domain of said PNS SCP.
3. An isolated nucleic acid molecule according to claim 2, wherein said domain is selected from the group consisting of at least one of amino acids 1-17, 229-258, 268-272, 304-325, 330-393, 474-478, 501-505, 550-559, 589-593, 611-615, 619-646, 676-682, 689-694 and 779-805 of SEQ ID NO:2.
- 10 4. A recombinant nucleic acid molecule comprising, 5' to 3',
  - (A) a promoter effective to initiate transcription in a host cell; and
  - (B) an isolated nucleic acid molecule according to claim 1.
5. A recombinant host, comprising a recombinant nucleic acid molecule according to claim 4.
- 15 6. An isolated sodium channel peptide, comprising a peripheral nervous system specific (PNS) sodium channel peptide (SCP) including an amino acid sequence of at least 20 amino acids having at least 91% homology with a corresponding amino acid sequence of SEQ ID NO:2.
7. An isolated peptide according to claim 6, wherein said SCP has sodium channel (SC) biological activity.
- 20 8. An isolated peptide according to claim 6, wherein said isolated peptide corresponds to at least one domain of a PNS SC.
9. An isolated peptide according to claim 8, wherein said domain is selected from the group consisting of at least one of amino acids 1-17, 229-258, 268-272, 304-325, 330-393, 474-478, 501-505, 550-559, 589-593, 611-615, 619-646, 676-682, 689-694 and 779-805 of SEQ ID NO:2.
- 25 10. An isolated nucleic acid probe for the detection of the presence of a PNS SCP encoding DNA in a sample, said probe comprising a nucleic acid molecule sufficiently complementary to said PNS SCP encoding DNA to specifically detect under highly stringent hybridization conditions the presence of an isolated nucleic acid according to claim 1 in said sample.
11. An isolated probe according to claim 10, wherein said probe is detectably labeled as a detectably labeled
- 30 probe.
12. A method of detecting PNS SCP encoding nucleic acid in a sample comprising:
  - (A) contacting said sample with a detectably labeled probe according to claim 11, under conditions such that hybridization occurs, and
  - (B) detecting the presence of said labeled probe bound to PNS SCP nucleic acid.
- 35 13. An antibody which binds an epitope specific for a peptide according to claim 6.
14. An antibody according to claim 13, wherein said antibody is a detectable antibody which is detectably labeled or which binds a detectable label.

15. A host cell which produces an antibody according to claim 13.
16. A method of detecting a PNS SCP peptide in a biological sample, comprising:
  - (A) contacting said sample with a detectable antibody according to claim 14, under conditions such that immunocomplexes form; and
  - 5 (B) detecting the presence of said detectable antibody which has been labeled and is bound to said peptide.
17. A bioassay for assessing a candidate modulating agent of a PNS SCP, comprising:
  - (A) contacting a candidate agent with a cell line expressing in the cell membrane of said cell a PNS SCP ; and
  - 10 (B) evaluating the modulation of the SC biological activity of said cell mediated by said contacting of said candidate agent.
18. A method according to claim 17, wherein said cell line is selected from PC12 cells or a recombinant form thereof having an isolated nucleic acid molecule according to claim 1.
19. A PNS SCP modulating agent, identified by a method according to claim 17.
- 15 20. A PNS SCP modulating agent according to claim 19, wherein said agent is a methyl-phenyl/halophenyl-substituted piperazine compound.
21. A PNS SCP modulating agent according to claim 20, wherein said piperazine compound is lidoflazine (Merck Index Monograph 5311) or a derivative thereof.
22. A method of treatment for a sodium channel-associated pathology or trauma in a mammal, comprising  
20 administering to said mammal a therapeutically effective amount of an therapeutic nucleic acid, comprising a nucleic acid molecule according to claim 1, or an antisense nucleic acid complementary thereto, provided in a gene delivery vector.
23. A method according to claim 22, wherein said treatment is for pain and said therapeutic nucleic acid is said antisense nucleic acid.
- 25 24. A pharmaceutical composition, comprising an isolated nucleic acid according to claim 1, or an antisense nucleic acid complementary thereto, and a pharmaceutically acceptable carrier.
25. A recombinant virion comprising an expression vector having an isolated nucleic acid according to claim 1, or an antisense nucleic acid complementary thereto.
26. A method to treat diseases or conditions mediated by the abnormally low level of expression or  
30 function of a PNS SCP comprising administering to a patient in need of such treatment an effective amount of an isolated nucleic acid according to claim 1.
27. A compound capable of binding to a sodium channel peptide according to claim 6 and modulating the SC activity of said peptide.
28. A pharmaceutical composition comprising a compound according to claim 27 and a pharmaceutically  
35 acceptable carrier.
29. A method to treat diseases or conditions mediated by the presence of a PNS SCP, comprising administering to a patient in need of such treatment an effective amount of a PNS SCP modulating agent according to claim 19, or a pharmaceutical composition thereof.

30. A method for providing a molecular model of a PNS SCP, comprising

(a) providing a computer readable medium having recorded thereon data corresponding to a coding sequence, a homologous amino acid or nucleic acid sequence, a structural domain or a functional domain of a PNS SCP comprising an amino acid or a nucleotide sequence of at least one PNS SCP, or at least one domain thereof;

5 (b) optionally providing a computer readable medium having recorded thereon x-ray diffraction data of said PNS SCP in crystalline form, said data sufficient to model the three-dimensional structure of said PNS SCP;

(c) analyzing on a computer the amino acid or nucleotide sequence data from (a) and optionally the x-ray diffraction data from (b), to provide data output defining a molecular model of at least one PNS SCP, or at least one domain thereof, said analyzing utilizing computing subroutines selected from the group consisting of data processing and reduction, auto-indexing, intensity scaling, intensity merging, amplitude conversion, truncation, molecular replacement, molecular alignment, molecular refinement, electron density map calculation, electron density modification, electron map visualization, model building, rigid body refinement and positional refinement; and

(d) obtaining atomic model output data defining the three-dimensional structure of said PNS SCP, or at least one domain thereof.

15 31. A computer readable medium having recorded thereon molecular model data of a PNS SCP as the model output data produced by a method according to claim 30.

32. A computer-based system for providing a molecular model of a PNS SCP, comprising the following elements;

(a) a computer readable medium having recorded thereon data corresponding to a amino acid or nucleotide sequence of at least one PNS SCP, or at least one domain thereof;

20 (b) optionally, a computer readable medium having recorded thereon x-ray diffraction data of said at least one PNS SCP or at least one domain thereof;

(c) at least one computing subroutine for analyzing on a computer the amino acid sequence data from (a) and optionally, the x-ray diffraction data from (b) to provide data output defining a molecular model of PNS SCP, or at least one domain thereof, said analyzing utilizing computing subroutines selected from the group consisting of data processing and reduction, auto-indexing, intensity scaling, intensity merging, amplitude conversion, truncation, molecular replacement, molecular alignment, molecular refinement, electron density map calculation, electron density modification, electron map visualization, model building, rigid body refinement and positional refinement; and

25 (d) retrieval means for obtaining model output data defining the three dimensional structure of said 30 PNS SCP, or at least one domain thereof..

33. A computer readable medium, comprising molecular model data of at least one PNS SCP produced by a method according to claim 32.

34. A method for providing a computer molecular model of a ligand of a PNS SCP, comprising

(a) providing a computer readable medium according to claim 33 comprising molecular model data 35 of a PNS SCP, or at least one domain thereof;

(b) providing a computer readable medium having recorded thereon molecular model data sufficient to generate molecular models of potential ligands of said PNS SCP;

- (c) analyzing on a computer the molecular model data from (a) and the ligand data from (b), to determine binding sites of said PNS SCP and to provide data output defining a molecular model of a ligand of said PNS SCP, said analyzing utilizing computing subroutines selected from the group consisting of data processing and reduction, auto-indexing, intensity scaling, intensity merging, amplitude conversion, truncation, molecular replacement, molecular alignment, molecular refinement, electron density map calculation, electron density modification, electron map visualization, model building, rigid body refinement and positional refinement; and
- (d) obtaining model output data defining a molecular model of at least one ligand of a PNS SCP, or a domain thereof.
- 10— 35. A PNS SCP ligand molecular model, comprising a computer readable medium having recorded thereon the model output data produced by a method according to claim 34.
36. An isolated PNS SCP ligand corresponding to the physical molecule of the molecular model of the ligand model produced by a method according to claim 34.
37. A computer-based system for providing a molecular model of a ligand of a PNS SCP, comprising the
- 15 following elements;
- (a) a computer readable medium having recorded thereon molecular model data of a PNS SCP, or at least one domain thereof;
- (b) a computer readable medium having recorded thereon molecular model data sufficient to generate molecular models of potential ligands of said PNS SCP;
- 20 (c) at least one computing subroutine for analyzing on a computer the molecular model data of said PNS SCP from (a) and the ligand data from (b), to determine binding sites of PNS SCP and to provide data output defining a molecular models of potential ligands of PNS SCP, said analyzing utilizing at least one computing subroutine selected from the group consisting of data processing and reduction, auto-indexing, truncation, molecular replacement, molecular alignment, molecular refinement, molecular translation, R-factor determination, electron density modification,
- 25 electron density mapping, map density averaging, map visualization, model building, rigid body refinement, position refinement, crystallographic water adding, geometrical analysis and B-factor averaging; and
- (d) retrieval means for obtaining model output data defining the molecular models of potential ligands of said PNS SCP.
38. A computer readable medium, comprising molecular model output data of a potential ligand of said
- 30 PNS SCP, said data produced by a method according to claim 37.
39. An isolated PNS SCP ligand, corresponding to the physical molecule of the molecular model of a ligand produced by a computer system according to claim 37.



PNI  
 Type II

ATAGTTGAACACAGCTGGTTTGAAGCTTCATCGTTCTCATGATCCTGCTCAGCAGCTGGAGCTCTGGCTTTTGAA 75  
 I V E H S W F E S F I V L H I L L S S G A L A F E  
 I V E H N W F E T F I V RESI

GATATCTATATTGAAAAGAAAAAGACCATTAAGATTATCTGGAGTATGCTGACAAGATATTCACCTACATCTTC 150  
 D I Y I E K K K T I K I I L E Y A D K I F T Y I F

ATTCGTGAAATGCTCTTAAATGGGTGGCATATGGGTATAAAACATATTTCACTAATGCCTGGTGTGGCTGGAC 225  
 I L E H L L K N W V A Y G Y K T Y F T N A W C W L D

TTCTTAATTGTTGATGTCTCTAGTACTTTAGTAGCCAACTCTTGGCTACTCAGACCTTGGCCCATTA 300  
 F L I V D V S L V T L V A N T L G Y S D L G P I K

TCTCTACGGACACTGAGGCGCCTAAGACCCCTAAGAGCCTTGTCTAGATTTTGAAGGAATGAGGCTAGTGCTCAAC 375  
 S L R T L R A L R P L R A L S R F E G M R V V V N  
 F E G M R V

GCACCTATAGGACCAATCCCTCCATCATGAACGTGCTTCTGCTGCTGCTTATATTTCTGGCTAATATTAGCATC 450  
 A L I G A I P S I M N V L L V C L I F W L I F S I

ATGGGAGTCAATCTGTTTGTGCGAAGTTCATGAGTGTGTCAACACCGAGTGGGTACGATTTCCTACATCT 525  
 M G V N L F A G K F Y E C V N T T D G S R F P T S

CAAGTTGCAAAACGTTCTGAGTGTTTTGCCCTGATGAACGTTAGTGGAAATGTGGAGTGGAAAAACCTGAAAGTA 600  
 Q V A N R S E C F A L M N V S G N V R W K N L K V

AACTCGACACAGCTGGGCTTGGTTACCTGTGCTGCTTCAAGTTGCAACATTCAGGGCTGGATGATATTATG 675  
 N F D N V G L G Y L S L L Q V A T F K G W H D I H

YATCGACGAGTTGACTCTGTTAATGTAAATGAACGCGGAATAAGAAACAGTCTCTACATGTACATTACTTT 750  
 Y A A V D S V N V N E Q P K Y E Y S L Y M Y I Y F

GTCATCTTCATCTCTTGGGCTCATCTTCACGTTGAACGTTTCATGCTGTCATCATAGATAATTTCAACCA 825  
 V I F I I F G S F F T L N L F I G V I I D N F N Q

CAGAAAAAAGCTTGGAGGTCAAGATATCTTTATGACAGAAAGACAGAAAGAAATACATATATGCAATGAAGAG 900  
 Q K K K L G G Q D I F M T E E Q K K Y Y N A M K K

CTTGGGTCCAAAAAACAACAAAAACAAATCCAAGGCCAGGGAACAAATCCAAGGATGTATATTGAC 969  
 L G S K K P Q K P I P R P G N K F Q G C I F D

Figure 1

1128

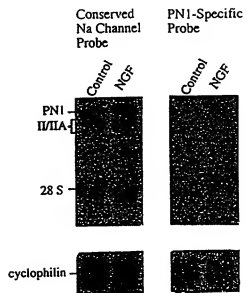
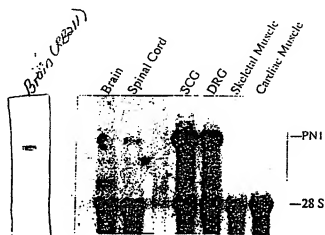


Figure 2

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3A



3B

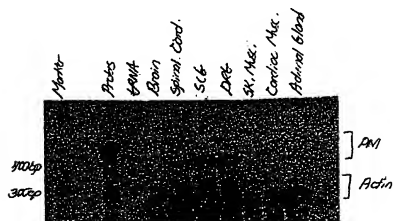


Figure 3A-B

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SCG

DRG

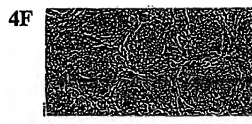
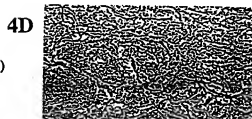
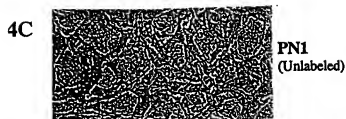
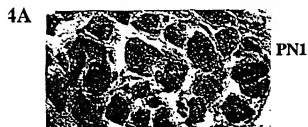


Figure 4A-F

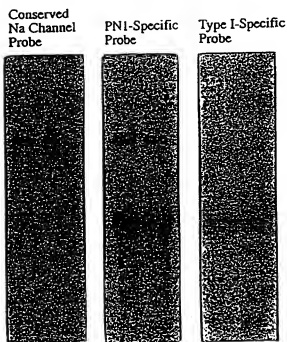
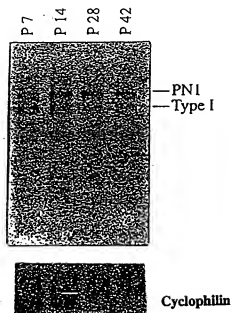


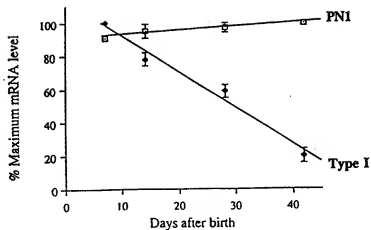
Figure 5

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6A



6B



$\left( \begin{array}{l} -3 \text{ points} \\ -1 \text{ point} \pm SE \end{array} \right)$

Figure 6A-B

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10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
AGGAACCTTG	TGGTCCGTGAA	CCGTGTTTCIG	GCCTCTTTTGC	TGAGTTCCCTT	50
R N L V	V L N L F L	A L L L S S F			
TAGTTCIGAC	AATCTTACAG	CAATTCAGGA	AGACACCGAT	GCAAACAACC	100
S S D N L T A	I E E D T D	A N N L			
TCCAGATCGC	AGTGGCCAGA	ATTAAAGGG	GAATCAATTA	CGTGAACAG	150
Q I A V A R	I K R G	I N Y V K Q			
ACCCIGGGTG	AATTCATCT	AAAATCAITTT	TCCAAAAAGC	CAAAGGGCTC	200
T L R E	F I L K S F	S K K P K G S			
CAAGGACACA	AAACGAACAG	CAGATCCCAA	CAACAAGAAA	GAAACTATA	250
K D T K R T A	D P N N K K	E N Y I			
TTTCAAAACG	TACCCCTTGCG	GAGATGAGCA	AGGATCACAA	TTTCTCTAAA	300
S N R T L A	E M S K	D H N F L K			
GAAAAGGATA	GGATCAGTGG	TTATGGCAGC	AGTCTAGACA	AAAGCTTTAT	350
E K D R	I S G Y G S	S L D K S F M			
GGATGAAAT	GATTACAGT	CCITTATCCA	TAAACCCAGC	CTCAGCTGA	400
D E N D Y Q S	F I H N P S	L T V T			
CAGTCCCAAT	TGCACCTGGG	GAGTCIGATT	TGGACATTAT	GAACACAGAA	450
V P I A P G	E S D L E I M N T E				
GAGCTTAGCA	GTCAGTCAGA	CAGTGACTAC	AGCAAGAGAA	AAACGAAACG	500
E L S S D S D	S D Y S K E K	R N R			
ATCAAGCTCT	TCGTAGTGA	GCATGTTGA	CAACCTTCIG	CCAGGAGAG	550
S S S S E C S	T V D N P L P G E E				
AGGAGCTIGA	AGCAGAGCC	GTAACCCAG	ATGAGCTIGA	AGCTGCTTT	600
E A E A E P	V N A D E P E A C F				
ACAGATGGTT	GTTGAGGAG	ATTTCATGC	TGCCAAGITA	ATGTAGACTC	650
T D G C	V R R F P C	C Q V N V D S			
TGGGAAGGG	AAAGTTTGGT	GGACCATCAG	GAAGAGCTGC	TACAGGATAG	700
G K G K V W W	T I R K T C Y R I V				
TTGAACACAG	CTGGTTTGA	AGCTTCATCG	TTCTCATGAT	CCTGCTCAGC	750
E H S W F E	S F I V L M I L L S				
AGTGGAGCTC	TGGCTTTTGA	AGATATCTAT	ATTGAAAAGA	AAAAGACCAT	800
S G A L A F E	D I Y I E K K K T I				
TAAATATATC	CTGGAGTATG	CTGACAAGAT	ATTCACTTAC	ATCTTCATTC	850
K I I L E Y A	D K I F T Y I F I L				
TGAAATGCT	TCTAAAATGG	GTCGCATATG	GGTATAAAAC	ATATTTCACT	900
E M L L K W	V A Y G Y K T Y F T				
AATGCTTGGT	GTTGGCTGGA	CTTCTTAATT	GTTGATGTTG	CTCTAGTTAC	950
N A W C W L D	F L I V D V S L V T				

Figure 7A

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10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
TTTAGTAGGC	AACACTCTTG	GCTACTCAGA	CCITGGCCCC	ATTAAATCTC	1000
L V A N T L G	Y S D L G P	I K S L			
TACGGCACT	GAGGCCCCA	AGACCCCTAA	GAGCCTTGTC	TAGATTTGAA	1050
R T L R A L	R P L R	A L S R F E			
GGAATGAGG	TAGTGGTCAA	CGCACTCATA	GGAGCAATCC	CTTCATCAT	1100
G M R V V V N	A L I G A I P	S I M			
GAACTGCTT	CTGTGTGGC	TTATATCTG	GCTAATATTT	AGCATCATGG	1150
N V L L V C L	I F W L I F	S I M G			
GAGTCAATCT	GTITGCTGGC	AAGTCTCATG	AGTGTGTCAA	CACCAACGAT	1200
V N L F A G	K F Y E	C V N T T D			
GGGTCAGAT	TTCCTACATC	TCAAGTTGCA	AACCGTCTG	AGTGTGTGGC	1250
G S R F P T S	Q V A N R S E	C F A			
CCITGATGAC	GTITGTGGAA	ATGTGCGATG	GAAAAACCTG	AAAGTAACT	1300
L M N V S G N	V R W K N L	K V N F			
TGACACAGT	TGGGCTTGGT	TACCTGTGCG	TGCTTCAAGT	TGCAACATTC	1350
D N V G L G	Y L S L	L Q V A T F			
AAGGGCTGGA	TGGATATAT	GTATGACCA	GTGTACTCTG	TTAATGTAAA	1400
K G W M D I M	Y A A V D S V	N V N			
TGAACAGCG	AAATACGAAT	ACAGTCTTA	CATGTACATT	TACTTTTGICA	1450
E Q P K Y E Y	S L Y M Y I	Y F V I			
TCITCATCAT	CTTGGCTCA	TTCCTACAGT	TGAACCTGTT	CATTTGGTGT	1500
F I I F G S	F F T L	N L F I G V			
ATCATAGATA	ATTTCACCA	ACAGAAAAA	AAGCTTGGAG	GTCAGATAT	1550
I I D N F N Q	Q K K K L G G	Q D I			
CTTTATGCA	GAAGAACAGA	AGAAATACTA	TAATGCAATG	AAGAAGCTTG	1600
F M T E E Q K	K Y Y N A M	K K L G			
GGTCCAAAA	ACCACAAAA	CCATTTCCAA	GGCAGGGAA	CAATTTCCAA	1650
S K K P Q K	P I P R	P G N K F Q			
GGATGTATAT	TTGACTTAGT	GACAAACAA	GCTTTTGATA	TCACCATCAT	1700
G C I F D L V	T N Q A F D I	T I M			
GGTCTCTATA	TGCTCAACA	TGTAACCAT	GATGGTAGAA	AAAGAGGGG	1750
V L I C L N M	V T M M V E	K E G Q			
AAACTAGTGA	CATGATTAT	GTITTACACT	GGATCAACAT	GGTCTTCAIT	1800
T E Y M D Y	V L H W	I N M V F I			
ATTCGTGTCA	CTGGGGAGTG	TGTCGTGAAG	CTAATCTCC	TCAGACATTA	1850
I L F T G E C	V L K L I S L	R H Y			
CTACTTCACT	TGCGGTGGA	ACATTTTGTA	TTTGTGGTGA	GTGATCTCT	1900
Y F T V G W N	I L Y F V V	V I L S			

Figure 7B

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10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
CCATTGTAGG	AATGTTTCTC	GCTGAGATGA	TAGAGAAGTA	TTTGTGTGTC	1950
I V G M F L	A E M I	E K Y	F V S		
CCTACCGTGT	TCCGAGTCAT	CCGCGTGGCC	AGGATTGGAC	GAATCCATAG	2000
P T L F	R V I	R L A	R I G R	I L R	
CCTGATCAAA	GGCGCAAGG	GGATCCGCAC	TCTGCTCTTT	GCCTTGTATGA	2050
L I K G A K G	I R T	L L F	A L M M		
TGTCCTCTCC	TGCGCTGTTC	AACATCGGCC	TCTGCTCTTT	CCCTGGTCATG	2100
S L P A L F	N I G L	L L F	L V M		
TTCATCTACG	CCATCTTTTG	GATGTCCAAC	TTTGCCCTACG	TTAAAAAGGA	2150
F I Y A I F G	M S N	F A Y V	K K E		
GGCTGGAAIT	AATGACATGT	TCAACTTTGA	GACTTTTGGC	AACAGCATGA	2200
A G I N D M F	N F E	T F G	N S M I		
TCTGCTGTGT	CCAAATCACC	ACCTCTGCGG	GCTGGGACGG	ACTGCTGGCC	2250
C L F Q I T	T S A G	W D G	L L A		
CCCATCTCCA	ACAGCGCACC	TCCCGACTGT	GACCTTAAAA	AAGTTCACCC	2300
P I L N S A P	P D C	D P K K	V H P		
AGGAAGTICA	GTTGAAGGGG	ACTGTGGGAA	CCCTTCGGTG	GGGATTTTTT	2350
G S S V E G D	C G N	P S V	G I F Y		
ACTTTGTICAG	CTACATCATC	ATATCTCTCC	TGGTGGTGGT	GAACATGTAC	2400
F V S Y I I	I S F L	V V V	N M Y		
ATGCTGTICA	TCTTGGAGAA	CTTCAGCGTC	GCCACCGAAG	AGACCATCTGA	2450
I A V I L E N	F S V	A T E E	S T E		
GCCTCTGAGT	GAGGACGACT	TTGAGATGTT	CTACGAGGTC	TGGGAGAAGT	2500
P L S E D D F	E M F	Y E V	W E K F		
TGGACCTTGA	CGCCACTCAG	TTCATAGAGT	TCTGCAAGCT	CTCTGACTTT	2550
D P D A T Q	F I E F	C K L	S D F		
GCAGCTGCC	TGGATCTCTC	CCTCTCTATC	GCAAAGCCAA	ACAAAGTCCA	2600
A A A L D P P	L L I	A K P N	K V Q		
GCTCATTTGC	ATGGACCTGC	CCATGGTGAG	TGGAGACCGC	ATCCCATGCC	2650
L I A M D L P	M V S	G D R	I H C L		
TGGACATCTT	GTITGCTTTT	ACAAAGCGGG	TCTTGGGTGA	GGGTGGAGAG	2700
D I L F A F	T K R V	L G E	G G E		
ATGGATTTCT	TTGTTTCACA	GATGGAGGAA	AGGTTCATGT	CAGCCATTC	2750
M D S L R S Q	M E E	R F M S	A N P		
TTCTAAAGTG	TCTTATGAAC	CCATCAGGAC	CACACTGAAG	AGAAAACAG	2800
S K V S Y E P	I T T	T L K	R K Q - E		
AGGAGGTGTC	CGGACTATC	ATTTCAGGGT	CTTACAGACG	GTTATCGCTC	2850
E V S A T I	I Q R A	Y R R	Y R L		

Figure 7C

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10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
AGACAACACG	TCAAGAATAT	ATCGAGTATA	TACATAAAAG	ATGGAGACAG	2900
R Q H V	K N I	S S I	Y I K D	G D R	
GGATGATGAT	TTGCCCAATA	AAGAAGATAC	AGTTTITIGAT	AACGIGAACG	2950
D D D	L P N K	E D T	V F D	N V N E	
AGAACTCAAG	TCCGGAAGAG	ACAGATGTAA	CTGCTCAAC	CATCTGCCCA	3000
N S S	P E K	T D V T	A S T	I S P	
CCTTCTATG	ACAGTGTAC	AAAGCCAGAT	CAA		3033
P S Y D	S V T	K P D	Q		

Figure 7D

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PN1 T	MARSVLVPPG	PDSFRFFFTRE	SLAAIEQRIA	EEKAKRPKQE	RKDEDDENG	50
RNSCPIIR T	Consensus	.....	.....	.....	.....	50
PN1 T	KPNSDLLEAGK	SLPFFIYGDI	PEMVSEPLED	LDPYYINKKT	FIVLNKGKAI	100
RNSCPIIR T	Consensus	.....	.....	.....	.....	100
PN1 T	SRFSATSALY	ILTPFNPIRK	LAIKILVHSL	FNVLMCTIL	TNCVPMTHSN	150
RNSCPIIR T	Consensus	.....	.....	.....	.....	150
PN1 T	PPDWTKNVEY	TFTGIYTFES	LIKILARGFC	LEDFTFLRNP	WNWLDFTVIT	200
RNSCPIIR T	Consensus	.....	.....	.....	.....	200
PN1 T	FAYVTEFVNL	GNVSALRTR	VLRAKLTISV	IPGLKTVGA	LIQSVKKLSD	250
RNSCPIIR T	Consensus	.....	.....	.....	.....	250
PN1 T	VMILTIVFCLS	VFALIGLQLF	MGNLRNKCLO	WPPDNSTFEI	NITTSFFNLSL	300
RNSCPIIR T	Consensus	.....	.....	.....	.....	300
PN1 T	DWNGTAFNRT	VNMFNWDEYI	EDKSHFYFLE	QONDALLCGN	SSDAGQCPEG	350
RNSCPIIR T	Consensus	.....	.....	.....	.....	350
PN1 T	YICVKAGRNP	NYGYTSDFTF	SWAPLSLPLR	HTQDFWENLY	QLTLRAAGKT	400
RNSCPIIR T	Consensus	.....	.....	.....	.....	400
PN1 T	YMFFVVLVIF	LGSFYLINLI	LAVVAMAYEE	QNQATLEEAE	QKEAEFQOML	450
RNSCPIIR T	Consensus	.....	.....	.....	.....	450
PN1 T	BQLKKQEEA	QAAAAASAE	SRDFSGAGGI	GVFSESSVA	SKLSSKSEKE	500
RNSCPIIR T	Consensus	.....	.....	.....	.....	500

**Figure 8A**

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**Figure 8B**

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PN1 T	-----	
RNSCPIIR T	LKNRRKKKKQ KEQAGEEEKE DAVRKSASED SIKRKGQFQS LEGSRLTYEK	550
Consensus	.....	550
PN1 T	-----	
RNSCPIIR T	RFSSPHQSLI SIRGSLFSPR RNSRASLFNF KGRVKDIGSE NDFADDEHST	600
Consensus	.....	600
PN1 T	-----	
RNSCPIIR T	FEDNDSRRDS LFVPHRHGER RPSNVQASR ASRGITLPM NGKMHSAVDC	650
Consensus	.....	650
PN1 T	-----	
RNSCPIIR T	NGVSVLVGGP SALTSPVGQL LPEGTTTETE IKRRSSSYH VSMDLLEDPS	700
Consensus	.....	700
PN1 T	-----	
RNSCPIIR T	RQRAMSMASI LTNMEELEE SRQKCPFCWY KPANMCLIND CCKPWLKVKH	750
Consensus	.....	750
PN1 T	-----	
RNSCPIIR T	VVNLVVMDFP VDLAITICIV LNTLFMAMEH YPMTBQFSSV LSVGNLVFTG	800
Consensus	.....	800
PN1 T	-----	
RNSCPIIR T	IFTAEMFLKI IAMDPIYYFO EGNIFDGF I VLSLSMELGL ANVEGLSVLR	850
Consensus	.....	850
PN1 T	-----	
RNSCPIIR T	SFRLLRVFKL AKSWPTLNL I KIIGNSVGA LGNLTVLVLI IVFIFAVVGM	900
Consensus	.....	900
PN1 T	-----	
RNSCPIIR T	QLPGKSYKEC VCKISNDCEL PRWIDGHHFH SFLIVEVLC GEMLETWDC	950
Consensus	.....	950
PN1 T	-----	
RNSCPIIR T	MEVAGQTMCL TVFMVMVIG	1000
Consensus	.....	1000

Figure 8C

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PN1 T	NNISQVAVARI KPSNVMVOT I RRRN	81
RNSCPIIR T	NNISQVAVARI KPSNVMVOT I RRRN	1050
Consensus	NNISQVAVARI KPSNVMVOT I RRRN	1050
PN1 T	NYISNVLAA MSKQVNRK RD-RISG	130
RNSCPIIR T	NYISNVLAA MSKQVNRK RD-RISG	1100
Consensus	NYISNVLAA MSKQVNRK RD-RISG	1100
PN1 T	NYISNVLAA MSKQVNRK RD-RISG	180
RNSCPIIR T	NYISNVLAA MSKQVNRK RD-RISG	1150
Consensus	NYISNVLAA MSKQVNRK RD-RISG	1150
PN1 T	NYISNVLAA MSKQVNRK RD-RISG	229
RNSCPIIR T	NYISNVLAA MSKQVNRK RD-RISG	1200
Consensus	NYISNVLAA MSKQVNRK RD-RISG	1200
PN1 T	NYISNVLAA MSKQVNRK RD-RISG	279
RNSCPIIR T	NYISNVLAA MSKQVNRK RD-RISG	1250
Consensus	NYISNVLAA MSKQVNRK RD-RISG	1250
PN1 T	NYISNVLAA MSKQVNRK RD-RISG	329
RNSCPIIR T	NYISNVLAA MSKQVNRK RD-RISG	1300
Consensus	NYISNVLAA MSKQVNRK RD-RISG	1300
PN1 T	NYISNVLAA MSKQVNRK RD-RISG	379
RNSCPIIR T	NYISNVLAA MSKQVNRK RD-RISG	1350
Consensus	NYISNVLAA MSKQVNRK RD-RISG	1350
PN1 T	NYISNVLAA MSKQVNRK RD-RISG	429
RNSCPIIR T	NYISNVLAA MSKQVNRK RD-RISG	1400
Consensus	NYISNVLAA MSKQVNRK RD-RISG	1400
PN1 T	NYISNVLAA MSKQVNRK RD-RISG	479
RNSCPIIR T	NYISNVLAA MSKQVNRK RD-RISG	1450
Consensus	NYISNVLAA MSKQVNRK RD-RISG	1450
PN1 T	NYISNVLAA MSKQVNRK RD-RISG	529
RNSCPIIR T	NYISNVLAA MSKQVNRK RD-RISG	1500
Consensus	NYISNVLAA MSKQVNRK RD-RISG	1500

Figure 8D

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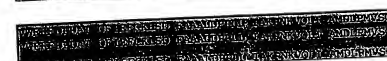

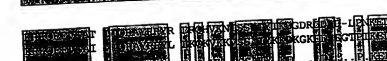
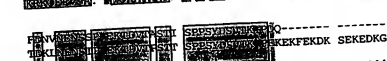
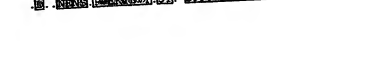

PNI T RNSCPIIR T Consensus											579 1550 1550
											629 1600 -1600
											679 1650 1650
PNI T RNSCPIIR T Consensus											729 1700 1700
											779 1750 1750
											829 1800 1800
PNI T RNSCPIIR T Consensus											879 1850 1850
											929 1900 1900
											978 1950 1950
PNI T RNSCPIIR T Consensus											1011 2000 2000
											1011 2000 2000
											1011 2000 2000

Figure 8E

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PN1 T -----  
RNSCPIIR T RESKK  
Consensus .....

1011  
2005  
2005

Figure 8F

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GTGCGCTCAT	CCTGAGCAGA	CTGGAAACAG	ACTCGGTGCA	GGCGTGGCCC	50
GGCGTCCAGT	TGCGAGCTGTA	GGGTTTTCAT	TCTTGCCGAC	TGCGGAGACT	100
GGCGTGGAGCT	AGCTTGGGTA	TCGAGGATTC	GCGACTCGTA	GTACACAGGCA	150
CTTGAGCAGA	CAGGATTTCA	GAGAAAGAG	CAGAGGCGAG	AAAGAAGCCT	200
GGGAGAGAG	GAGACTTTTC	CTTGGATCAG	ACTCGCGAGG	TGCAACACAC	250
GGGTGGGCAT	GATCCGTGGG	CGCAGGCCTC	TTAGGTAAAG	AGTCAAGAGG	300
GAATAAAGC	ATACAGGATG	AAAGAGTGGC	GATGCTGCCT	CCTCCAGGAC	350
CTCAGAGTTC	CGTTCACCTC	ACAAAACAGT	CCCTTGCCCT	CATTGAACAG	400
CGTATTTCTG	AGAAAAAGC	CAAGGAACAC	AAAGACGAAA	AGAAAGATGA	450
TGAGGAAGAA	GGCCCCAAGC	CCAGCAGTGA	CTTGGAGCT	GGGAAACAGC	500
TCCCTCTCAT	CTATGGAGAC	ATTCCCCCTG	GAATGGTGTG	AGAGCCCCCT	550
GAGGACCTGG	ACCCATACTA	TGCTGCACAA	AAAACTTTTA	TAGTATTGAA	600
CAAGGGGAAA	GGAATCTTCC	GTTTCAACGC	CACCCCTGCT	TTGTACATGC	650
TGCTCTCCCT	CAGTCCCTCA	AGAGAATAT	CTATTAGAT	CTTAGTGAC	700
TCCTTATTCA	GCACTGCTAAT	CATGTGCACA	ATTCTGACGA	ACTGCATATT	750
CATGACCTTG	AGCAACCCCT	CAGAATGGAC	CAAAAATGTA	GGGTACACTT	800
TTACTGGGAT	ATATACTTTT	GAATCACTCA	TAAAAATCCT	TGCAAGAGGC	850
TTTTTGGTGG	GAGAAATTCAC	CTTCTCCGT	GACCTTGGA	ACTGGCTGGA	900
ATGTTTTCAG	TCTTGGAACT	TTTTCAGTCT	AGAACTTGT	AACTAGGCA	950
TCTGTAAATC	CAGGACTTAA	GACCATCTG	GGGGCCCTGA	TCCAAGTATT	1000
GAAGAAGCTC	TCTGAGCTCA	TGATCTCTAC	TGTGTCTGT	CTCAGTGTGT	1100
TGTCATTAAT	TGGAATCAAG	CTGTTTATGG	GCAACTTGAA	GCATAAATGT	1150
TTGAGAGAG	AACCTGAAAG	GAATGAAACA	TTAGAAAGTA	TCATGAATAT	1200
TGCTGAGAGT	GAAGAAGAA	TGAAAATAA	TTTTTATTAC	TTGAGGGATC	1250
CCAAAGATGC	TCTACTCTGC	GGCTTCAGCA	CAGATTACGG	CGAGTGTCCA	1300
GAAGGCTACA	TCTGTGTGAA	GGCTGGCAGA	AACCGGAT	ATGGCTACAC	1350
GAGCTTTGAC	ACATTCACTG	GGGCTCTTCT	GGCCTTGTGT	CGGCTAATGA	1400
CTCAGGACTA	CTGGGAGAAC	CTTTACCAAC	AGACTCTGCG	TGCTGCTGGC	1450
AAAACCTACA	TGATTTTCTT	TGTCGTGTTT	ATTTTCTGCG	GCTCCTTTTA	1500
CCTGATAAAC	TTGATCTGGG	CTGTGGTAGC	CATGGCGTAT	GAGGAACAGA	1550
ACCAGGCCAA	CATCGAAGAA	GCTAAACAGA	AAAGTATAGA	ATTTCAAGCA	1600
ATGTTAGACC	GACTCAAAAA	GGAGCAGGAA	GAAGCTGAGG	CGATCGCTGC	1650
AGCTGCTGCT	GAGTTCAACGA	GTAATGGGCG	GAGCAGGATC	ATGGGACTCT	1700
CTGAGAGCTC	TTGAGAAACC	TCAGGCTGA	GCTCAAGAG	TGCCAAGAG	1750
AGAAGAAACC	GAAGAAGAA	AAAGAAGAG	AGATGTGCTA	GTGGCAGGGA	1800
AAAGGGTGA	GATGAGAAGC	TGTCGAGTC	AGGATCAGAG	GAAGACATC	1850
GAAGAAGAG	CTTCCATCTC	GGTGTGGAAG	GGCACACCG	GACCCCGGAA	1900
AAAGGCTGT	CCACCCGCCA	CCAGTCCGAG	CTCCTTCAGT	CGGGTCCCT	1950
GTTTTCTGCC	AGGCGGCACT	GAGCAGCAAT	TGCTGTATGA	TGAGCATAGC	2000
GAGGAAGAGA	TCTGGGATCT	ACAAACGAG	CAGAGGGGT	TCATATTG	2050
ATTTTGTGAG	CGGGCAGCA	GTAACTCAG	TCAGGCCAGT	AGGTCCCCGC	2100
ACCCGCGAG	CGGTGACGAG	AGATGACCA	GTGCACTGCA	CTGCAATGGA	2150
CAGTGTCTAC	GGTGAACGGG	ACCCCTCAGC	CTCATGTCC	CGAATGGACA	2200
GTCTGTGCTC	TTGTGTATGG	AGCTTCCGAG	AACCTCCGAG	GACAGCGGCA	2250
CGTTCTTCCA	GAGGTGATAA	TAGATAAGGC	CTAGTTCTTA	CTTCTGTCT	2300
CGACTAATCA	GATGCGCAAA	AAAAGGCTCT	CTAGTTCTTA	CTTCTGTCT	2350
GAGGACATGC	TGAATGACCC	GCATCTCAGG	CAAGGGGCCA	TGAGCAGGGC	2400
GAGCATACTG	ACCAACACTG	TGGAGAACT	TGAAGAATCT	AGACAAAAAT	2450
GTCAACAGTT	GTGTACAGA	TTTGTCAACA	CATTTTTAA	CTGGAATTGC	2500
TCCTCATATT	GGATAAATTT	CAAAAAGCTC	ATCTATTTTA	TTGTGATGGA	2550
TCCTTTTGTG	GATCTTGGAA	TTACCAATTG	CATGATTTTA	AACACTTAT	2600
TTATGGCTAT	GGAGCACAC	CCAATGACTG	AGAAATTCAA	AAATGTCCCT	2650
CGAGTGGGGA	ACTGTATCTT	TACAGGGATC	TTGCGAGCTG	AAATGTGACT	2700
GAAGTTAATA	GCCATGGACC	CTGATGAGTA	TTTCCAGTGA	GGGTGGAA	2750
TTTTTGTAGC	CCTAATTGTG	ACGCTGAGTT	TGATAGACT	TTTCTAGCA	2800
GATGTGGGAG	GATTATCAGT	TCGTCGGTCA	TTGAGATTC	TCCGAGTCTT	2850
CAGTGTGGCA	AAATCTGTGC	GGCACTGGA	CATGCTATT	AGATCATCTG	2900
GCAACTCGGT	GGGCTGTG	GGCAACTGGA	CCCTGTGCTG	GGCAATCATC	2950
GTCTTCTATT	TTGCTGTG	CGGCATGCG	CTGTTGGGAA	AGAGTACAAA	3000
GGAGTGTGCT	TGCAAGATCA	ATGTGGACTG	CAGCTGCGCG	CGCTGGCACA	3050
TGAACGACTT	CTTCACTCC	TTCTCATG	TGTTCCGAGT	GCTGTGTGGG	3100
GAGTGGATAG	AGACCATGTG	GGACTGCATG	GAGGTGCGCG	GGCAGACCAT	3150
GTGCTTATT	GTTTACATGA	TGCTCATGTT	GATTGGGAAAC	CTTGTGTGCC	3200
TGAACCTGTT	TCTGGCTCTT	TTGCTGAGTT	CCTTTAGTTC	TGCAATCTTT	3250
ACAGCAATGT	AGGAAGACAC	CGATGCAAAC	AACTCCGAGA	TGCGAGTGGC	3300
CAGAATTAA	AGGGGAATCA	ATTACGTGAA	ACAGACCTGT	GTGGAATTTCA	3350
TTCTAAATAT	ATTTTCAAAA	AAAGCCAAAG	GCTCAAGGA	CACAAAACGA	3400

FIGURE 9A  
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ACAGCAGATC	CCAACAACAA	GAAGAAAAAC	TATATTTCAA	ACCGTACCCT	3450
TGCGGAGATG	AGCAAGGATC	ACAATTTCCT	CAAGAAAGAG	GATAGATACA	3500
GTGGTTATGG	CAGCAGCTTA	GACAAAGGCT	TTATGGATAG	AAATGATTAC	3550
CAGTGCCTTA	TCCATAACCC	CAGCTTCACA	GTGACAGTGC	CAATTGCACC	3600
TGGGGAGTCT	GATTTGGAGA	TTATGAACAC	AGAAGAGCTT	AGCAGTGACT	3650
CAGACAGTGA	CTACAGCAAA	GAGAAACGGA	ACCGATCAAG	CTCTCTGAG	3700
TGCGACACTG	TTGACAACCC	TCTGCCAGGA	GAAGAGGAGG	CTGAGACAGA	3750
GCCCGTAAAC	GCAGATGAGC	CTGAAAGCCTG	CTTTACAGAT	GTTTGTGTGA	3800
GGAGATTTC	ATGCTGCCAA	GTTAATGTAG	ACTCTGGGAA	AGGGAAAGTT	3850
TGGTGGACCA	TCAGGAAGAC	GTGCTACAGG	ATAGTTGAAC	ACAGCTGGTT	3900
TGAAGGCTTC	ATCGTTCTCA	TGATCCTGCT	CAGCAATGGA	GCTCTGGCTT	3950
TTGAAGATAT	CTATATTGAA	AAGAAAAAGA	CCATTAAAGT	TATCCTGGAG	4000
TATGCTGACA	AGATATTAC	CTACATCTTC	ATTCTGGAAA	TGCTTCTAAA	4050
ATGGGTGCGA	TATGGGTATA	AAACATATTT	CACATAATGC	TGGTGTGGC	4100
TGGACTTCTT	AATTGTGTAT	GTGTCTTAG	TTACTTTAGT	AGCCACACT	4150
CTTGCTACT	CAGACCTTGG	CCCCATTAAA	TCTCTACGGA	CAGTGAGGCG	4200
CCTAAGACCC	CTAAGAGCCT	TGTCTAGATT	TGAAGGAATG	AGGGTAGTGG	4250
TCACGCACT	CTAGGAGACA	ATCCCTTCCA	TCATGAACGT	GCTTCTCTG	4300
TGCTTATAT	TCTGGCTAAT	ATTTAGCATC	ATGGAGATCA	ATCTGTTTGC	4350
TGCGAAGTTC	TATGATGTG	TCACACAC	CTATGGGTCA	CGATTCTCTA	4400
CATCTCAAGT	TGCAACATCT	TCTGAAGTGT	TTGGCCCTGAT	GAACCTTAGT	4450
GGAAATGTC	GAATGAAAAA	CCCTGAAGTA	AACCTGACCA	ACGTTGGGCT	4500
TGGTTACCTG	TGCGCTCTTC	AAGTTGCAAC	ATTCAAGGCG	TGGATGGATA	4550
TTATGTATGC	AGCAGTTGAC	TCTGTTAATG	TAAATGAACA	GCGGAAATAC	4600
GAATACAGTC	TCTACATGTA	CATTACTCTT	GTCACTCTCA	TGATCTTGGG	4650
CTCATCTCTC	ACGTTGAACC	TGTTCTATGG	TGTCATCATA	GATAATTTCA	4700
ACCAACAGAA	AAAAAAGCTT	GGAGGTCAAG	ATATCTTTAT	GACAGAAGAA	4750
CAGGAAGAA	ACTATAATGC	AATGAAGAAG	CTTGGGTCCA	AAAAACCAAC	4800
AAAAACCAAT	CCAAGGCCAG	GGAAACAATT	CCAGGATGAT	ATATTTGACT	4850
TAGTGAACAA	CCAAGCTTTT	GATATCACCA	TCATGTTCTT	TATATGCTCT	4900
AACATGGTAA	CCATGATGTT	AGAAAAAGAG	GGGCAAACTG	AGTACATGGA	4950
TTATGTTTTA	CAGTGGATCA	ACATGGTCTT	CATTATCCTG	TTCACTGGGG	5000
AGTGTGTGCT	GAAGCTAATC	TCCCTCAGAC	ATTACTACTT	CAGTGTGGGT	5050
TGGAACATTT	TGATTTTGTG	GGTAGTGATC	CTCTCCATTG	TAGGAATGTT	5100
TCTCGCTGAG	ATGATAGAGA	AGTATTTGTT	TGCCCTTACC	CTGTTCCGAG	5150
TCTCCGCTGC	GGCCAGGATT	GGACGAAATC	TACGCTGATG	CAAGGCGGCC	5200
AAGGGGATCC	GCACCTCTGCT	CTTGCTTTTG	ATGATGTGCC	TTCTCCGCTC	5250
GTCTCAACATC	GGCCTCTGTC	TTTTCTTGTT	CATGTTTATC	TACGCCATCT	5300
TTGGGATGTC	CAACTTTGCG	TACGTTAAAA	AGGAGGCTGG	AAATTAAGAT	5350
ATGTTCAACT	TAGTACACTT	GGCAACAGC	ATGATCTGCT	TGTTCCAACT	5400
CACCACTCTC	CCGCGCTGGG	ACCGACTGCT	GGCCCCATC	CTCAACAGCG	5450
CACCTCCGGA	CTTGACCTCT	AAAAAAGTTC	ACCCAGGAAG	TTCAAGTGGAA	5500
GGGACCTGTG	GGAACCCATC	CGTGGGGATT	TTTTACTTTG	TGAGCTACAT	5550
CATCATATCC	TTCTCGTGAG	TGGTGAACAT	GTACATCGCT	GTCACTCTGG	5600
AGAACTTCAG	CGTGCACC	GAAGAGAGCA	CTGAGCCTCT	GAGTGAAGGAC	5650
GACTTTGAGA	TGTTCTACGA	GCTCTGGGAG	AAGTTGAGCC	CTGACGCCAC	5700
TCAGTTTCATA	GAGTTCTGCA	AGCTCTCTGA	CTTAGCAGCT	GCCCTGGATC	5750
CTCCCCCTCT	CATCGCAAG	CCAAACAAAG	TCCAGCTCAT	TGCCATGGAC	5800
CTGCCCATGG	TGAGTGGAGA	CCGCACTCAC	TGCCGTGACA	TCCTTGTTCG	5850
TTTTACAAAG	CGGCTCTGG	GTGAGGGTGG	AGAGATGGAT	TCTCTTCGTT	5900
CACAGATGGA	AGAAAGGTTT	ATGTCAGCCA	ATCCTTCTAA	AGTGTCCCTAT	5950
GAACCCATCA	CGACCACTAT	GAAGAGAAAA	CAAGAGGAGG	TGTCGCGAAC	6000
TATCATTGAG	GTGCTTACA	GAGCGTATCG	CCTCAGACAA	CAGCTCAAGA	6050
ATATATGGAG	TATATACATA	AAGAGATGGG	ACAGGGATGA	TGATTTGCC	6100
ATAAAGAGAG	ATACAGTTT	TGATACGTTG	CAAGGAGACT	CAAGTCGGA	6150
AAGACAGAGT	GTAACTGCTT	GAACCATCTC	GCCACCTCTC	TATGACAGTG	6200
TCACAAAGCC	AGATCAAGAG	AAATATGAAA	CAGACAAAC	AGAGAAGGAA	6250
GACAAAGAGA	AGATCAAGAG	CAGGAATATG	AGCTTTGGTT	TTGATACACT	6300
CTTACAGCCG	TGTGAAGTTT	GACTCACTCG	TGTTAGTAGT	ACTCTTTTAC	6350
GGAGGTCTAT	CCAACTCTT	TTATCAAAAA	TTCTCAAGGC	AGCACAGCCA	6400
TTAGCTCTGA	TCCAAGAGG	CAGAGGCGAG	CATTATACAG	TGGCTATGTT	6450
TT					6452

FIGURE 9B

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NAMLPPPGPQ	SFVHFTKQSL	ALIEQRISSE	KAXEKKDEKK	DDEEGPKPS	50
SDLEAGKQLP	FYIGDIPPGM	VSEPLEDLDP	YYADKKTFFV	LNGKXAIFR	100
NATPALYMLS	PSPPLRIRSI	KILVHSLFSM	LIMCTILTNC	IFMTLSNPPE	150
WTKNGVYTF	GIYTFESLIK	ILARGFCVGE	FTFLRDPWNW	LOFVVIVFAY	200
LTFEVLNGVY	SALRTFRVLK	ALKTISVIPG	LKTIVGALIQ	SVKLSDVMI	250
LTVPCLSVFA	LIGLQLFMGN	LKHKCFRKL	EENETLESIM	NTAESEELK	300
KYFTYLEGSK	DALLCGFSTD	SGQCPEGYIC	VKAGRNPDPG	YTSFDTFSWA	350
FLALFRLMTQ	DYWNLYQQT	LRAAGKTYMI	FFVVVIFLGS	FYILINILAV	400
VAMAYEQNG	ANIERAKQKE	LEFOQMLDRL	KKEQEAEZAI	AAAAAEFTSI	450
GRSRIMGLSE	SSSETSRLLS	KSAKERNNRR	KKKQKMSG	EEKGDDEKLS	500
KSGSEESIRK	KSFHLGVEGH	HRTREKRLST	PNQSPLSIRG	SLFSARKESSR	550
TSLSFSKGRG	RDLGSETEFA	DDEHSIFGDN	ESRKGSLFVP	HRPRKRRSSN	600
ISQASRSPPV	LPVNGKMHSA	VDCNGVSVLV	DPSALMLPW	GQLLPEVIID	650
KATSDDSGTT	NQMRKKRLSS	SYFLSEDMAN	DPHLRGRAMS	RASILANTVE	700
ELEESRKQCH	QLIYRFARTF	LWKKSPYMI	KFKLLIVTIV	MDPPVDLAI	750
ICIVLNTILF	AMEHNPWTEE	FKNVLAVGNL	IFTGIFAEM	VLKLAMPDPY	800
EYFOGWNITP	DSLIVTLLSL	ELFLADVEGL	SVLESFRLR	VFKLAKSNPT	850
LWMLIKIION	SVGALGNLTL	VLAIIVPIFA	VVMQMLFGKS	YKECVCKINV	900
DCKLPRWMBN	DFPHSLIVF	RVLGEMMET	MWDOMEVAGO	TMCLIVYPMV	950
MVIGNLVVLN	LFJALLSSSF	SSDNLTAIEE	DTDANNLQIA	VARIKRGINY	1000
VKQTLREFIL	KSFSKFKPGS	KDKRTADPN	NKKENYISNR	TLAEMSKDHN	1050
FLKEKDRISG	YGSSLDKSPM	DENDYQSFH	NPSLTVTPVI	APGESDLEIM	1100
NTEELGSDSD	SDYSKEKRNK	SSSCESTVD	NPLPGEAEAE	AEPVNADEPE	1150
ACPTDGCVR	FPCCQVNVDS	GKGKVMNTIR	KTCYRIVEHS	WFESFIVLMI	1200
LLSSGALAFE	DIYIEKKKTI	KIILEYADKI	FTYIFILEML	LKNVAYGYKT	1250
YFTNANWCLO	FLIVDVSLVT	LVANTLGYSD	LGPISLRLT	RALRPLRLALS	1300
RFBGMRVVVN	ALIGAIPSIM	NVLVLCLIFW	LIFSIMGVNL	FAGKPYECVN	1350
TTDGRSPPTS	QVANRECEFA	LMSVSGNVNR	KHLKVMFNDV	GLGYLSLLQV	1400
ATFKGNMDIM	YAAVDSVNVN	EQPKYEYSLY	MYIYVFIFII	FOSFPTLNLF	1450
IGVIIDNPNQ	QKKKLGQDQI	FMTEEQKKYY	NAMKGLGSKK	PQRPIPRPN	1500
KFGQICFDLV	TNOAFDITIM	VLICLNMVTM	MVEKEQGTET	KDVLWNIIM	1550
VFIILFTGEC	VLKLISLRHY	YFTVGMNILY	FVVVILSLVO	NFLASHMIEKY	1600
FVSPTLFRVI	RLARIGRIKL	LKIGANGIRT	LLFALAMELF	ALPNIQLLLF	1650
LVMFIYAIIP	MSNFAIYKKE	ACINOWENTE	TFGNSMICLF	QITTSAGMDG	1700
LLAPIILNSAP	PDCQKVKVP	GSSVEGDCGN	PSVGIFVFSV	YIIISFLVVV	1750
NMYIAVILEN	FSVATEESTP	PLSKDDFEMF	YEVNEKFPDP	ATQFIEFCKL	1800
SDFAALDDP	LLIAKPNKQV	LIAMDLPMVS	GORHLCLOIL	PAFTKRVLGE	1850
QGBMDSLRSQ	MEERPMSANP	SKVSYPEITT	TLKRXKEEVS	ATIQRAYRR	1900
YRLRQHVXNI	SSIIYKDGDR	DDDLPNKEDT	VFDNVNNESS	PERTDVTAST	1950
ISPPSYDSVT	KPDQEKETD	KTEKEDKEKD	ESRK		1984

FIGURE 10

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RATPN1 610 VLPVNGI SAVDCNGVSVLDGPSALMLPNGQLLPEVIIDKATSDSDSG AQMRKRLSS
|||||
HUMPN1A XLPVNGKMHSAVDCNGVSVLDGKSALMLPNGQLLPEXXXXXXXXXXGTTNQXKHKRXS
HUMPN1B MLPVNGKMHSAVDCNGVSVLDGKSALMLPNGQLLPE-----GTTNQIHKHKRCS
HUMPN1C -LPVNGKMHSAVDCNGVSVLDG- SALMLPNGQLLPE-----GTTNQ--KCR--S
HUMPN1D VLPVNGKMHSAVDCNGVSVLDGPSALMLPNGQLLPEVIIDKATSDSDSGTTNQMKRKLSS

RATPN1 671 SYFLSEDMLNDPHLQRAMSRASILNTVTVEELESROKCHQLLYRFAHTFLIWNCSPPYWK
|||||
HUMPN1A SYFLSEDMLNDPHLQRAMSRASILNTVTVEELESROKCHQLLYRFAHTFLIWNCSPPYWK
HUMPN1B SYFLSEDMLNDPHLQRAMSRASILNTVTVEELESROKCPWYRFAHFKFLIWNCSPPYWK
HUMPN1C SY-LSSEDMLNDP-LRQRAMSRASILNTVTVEELESROK---YRFAH-FLIWNCSPPYWK
HUMPN1D SYFLSEDMLNDPHLQRAMSRASILNTVTVEELESROKCHQLLYRFAHTFLIWNCSPPYWK

RATPN1 732 FKKKIYFIVMDPFVDLAITICIVLNTLPMAMEHHPMTEEFKNVLAVGNLIFTGIFAAEMVL
|||||
HUMPN1A FKKKIYFIVMDPFVDLAITICIVLNTLPMAMEHHPMTEEFKNVLAXGNLXFTGIFAAEMVL
HUMPN1B FKKKIYFIVMDPFVDLAITICIVLNTLPMAMEHHPMTEEFKNVLAIIGNLXFTGIFAAEMVL
HUMPN1C FKK-IYFIVMDPFVDLAITICIVLNTLPMAMEHHPMTEEFKNVLA-GNL-FTGIFAAEMVL
HUMPN1D FKKKIYFIVMDPFVDLAITICIVLNTLPMAMEHHPMTEEFKNVLAVGNLIFTGIFAAEMVL

RATPN1 793 KLIAMDPEYFQVQWNI FDSLIVTLSLIELFLADVEGLSVLRSFLLRVFLKAKSWPTLNM
|||||
HUMPN1A KLIAMDPEYFQVQWNI FDSLIVTLSLIELFLADVEGLSVLRSFLLRVFLKAKSWPTLNM
HUMPN1B KLIAMDPEYFQVQWNI FDSLIVTLSLIELFLADVEGLSVLRSFLLRVFLKAKSWPTLNM
HUMPN1C KLIAMDPEYFQVQWNI FDSLIVTLSL-ELFLADVEGLSVLRSFLLRVFLKAKSWPTLNM
HUMPN1D KLIAMDPEYFQVQWNI FDSLIVTLSLIELFLADVEGLSVLRSFLLRVFLKAKSWPTLNM

RATPN1 854 LIKIIGNSVGAIGNLTLVLAIVFIFAVVGMLPGKSYKECVCKINVDCKLPRWRHNDFFH
|||||
HUMPN1A LIKIIGNSVGAIGNLTLVLAIVFIFAVVGMLPGKSYKECVCKINXDCKLPRWRHNDFFH
HUMPN1B LIKIIGNSVGAIGNLTLVLAIVFIFAVVGMLPGKSYKECVCKINDDCKLPRWRHNDFFH
HUMPN1C LIKIIGNSVGAIGNLTLVLAIVFIFAVVGMLPGKSYKECVCKIN-DC-LPRWRHNDFFH
HUMPN1D LIKIIGNSVGAIGNLTLVLAIVFIFAVVGMLPGKSYKECVCKINVDCKLPRWRHNDFFH

RATPN1 915 SFLIVFRVLGGEWIEITMWDMEVAGQTMCLIVYMMVMVIGNLVVLANFLALLSSPSSDNL
|||||
HUMPN1A SFLIVFRVLGGEWIEITMWDMEVAGQTMCLIVYMMVMVIGNLVVLANFLALLSSPSSDNL
HUMPN1B SFLIVFRVLGGEWIEITMWDMEVAGQAMCLIVYMMVMVIGNLVVLANFLALLSSPSSDNL
HUMPN1C SFLIVFRVLGGEWIEITMWDMEVAGQ-MCLIVYMMVMVIGNLVVLANFLALLSSPSSDNL
HUMPN1D SFLIVFRVLGGEWIEITMWDMEVAGQTMCLIVYMMVMVIGNLVVLANFLALLSSPSSDNL

RATPN1 976 TAIEEDTDANMLQIAVARIKRGINYVKQTLREFILKSPKPKGSKTKRTADPINKKENY
|||||
HUMPN1A TAIEEDTDANMLQIAVRIKKGINYVKQTLREFILKSPKPKGSKTKRTADPINKKENY
HUMPN1B TAIEEDTDANMLQIAVTRIKKGINYVKQTLREFILKSPKPKKISREIRQAEDLTKKENY
HUMPN1C TAIEED-DANMLQIAV-RIK-GINYVKQTLREFILK-PSKPKR-S-----D-N-KKENY
HUMPN1D TAIEEDTDANMLQIAVARIKRGINYVKQTLREFILKSPKPKGSKTKRTADPINKKENY

RATPN1 1037 ISNRTLAEMSKDNFLKEKDRISGYGSSLDKSPMDENDYQSF IHNPSLTVTVP IAPGESDL
|||||
HUMPN1A ISNRTLAEMSKGNFLKEKDRISGXGSSXDKGKGGGGGGQSGF IHNPSLTVTVP IAPGESDL
HUMPN1B ISNRTLAEMSKGNFLKEKDRISGFGSSVDKHLMESSDQSGF IHNPSLTVTVP IAPGESDL
HUMPN1C ISN-TLAEMSK-HNFLKEKD-ISG-GSS-DK--D--QSF IHNPSLTVTVP IAPGESDL
HUMPN1D ISNRTLAEMSKDNFLKEKDRISGYGSSLDKSPMDENDYQSF IHNPSLTVTVP IAPGESDL

RATPN1 1098 EIMNTEELSSDSDSDSYSEKKNRSSSSSECTVDNPLPGE EBAEAPVNADEPAECTDGC
|||||
HUMPN1A EXNKEELSSDSDSYSKXGNRSSSSSECTVDNPLPGE EBAEAPXNNDPEPAECTDGC
HUMPN1B ENNKEELSSDSDSYSKVLRNRSSSSSECTVDNPLPGE EBAEAPMNSDEPAECTDGC
HUMPN1C E-MN-EELSSDSDS-YSK---NRSSSSSECTVDNPLPGE EBAEAP-N-DEPAECTDGC
HUMPN1D EIMNTEELSSDSDSYSEKKNRSSSSSECTVDNPLPGE EBAEAPVNADEPAECTDGC

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FIGURE 11B

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RATPN1 1158 VRRFP: /NVDSGGKGVMTIRKTCYRIVEHSWFESFIVMLILLSSGQ AFEDIYIEKKK  
 |||||  
 HUMPN1A VRRFXCCOVNKSXSGKGQWNRKIRKTCYRIVEHSWFESFIVMLILLSSGALAFEDIYIEKKK  
 HUMPN1B VRRFSCCOVNIESGGKGVMTIRKTCYRIVEHSWFESFIVMLILLSSGALAFEDIYIEKKK  
 HUMPN1C VRRF -CCOVN -SGGK -WM -IRKTCY -IVEHSWFESFIVMLILLSSGALAFEDIYIE -KK  
 HUMPN1D VRRFPCCOVNVDSGGKGVMTIRKTCYRIVEHSWFESFIVMLILLSSGALAFEDIYIEKKK

RATPN1 1219 TIKIILEYADKIPTYIPILEMLLKWAYGYKTYPTNACWCLDFLIVDVS/LTVANTLGYS  
 |||||  
 HUMPN1A TIKIILEYADKIPTYIPILEMLLKWAYGYKTYPTNACWCLDFLIVDVS/LTVANTLGYS  
 HUMPN1B TIKIILEYADKIPTYIPILEMLLKWAYGYKTYPTNACWCLDFLIVDVS/LTVANTLGYS  
 HUMPN1C TIKIILEYADKIPTYIPILEMLLKWAYGYKTYPTNACWCLDFLIVDVS/LTVANTLGYS  
 HUMPN1D TIKIILEYADKIPTYIPILEMLLKWAYGYKTYPTNACWCLDFLIVDVS/LTVANTLGYS

RATPN1 1280 DLGPIKSLRTLRLALPLRALSRFEGMRVVVNALIGAIPSIHNVLLVCLIFWLIFSIMGVNL  
 |||||  
 HUMPN1A DLGPIKSLRTLRLALPLRALSRFEGMRVVVNALIGAIPSIHNVLLVCLIFWLIFSIMGVNL  
 HUMPN1B DLGPIKSLRTLRLALPLRALSRFEGMRVVVNALIGAIPSIHNVLLVCLIFWLIFSIMGVNL  
 HUMPN1C DLGPIKSLRTLRLALPLRALSRFEGMRVVVNALIGAIPSIHNVLLVCLIFWLIFSIMGVNL  
 HUMPN1D DLGPIKSLRTLRLALPLRALSRFEGMRVVVNALIGAIPSIHNVLLVCLIFWLIFSIMGVNL

RATPN1 1341 FAGKPYECVNTTSGSRFPTSQVANRSECFALMNVSGNVRWKLKVNFDNVGLGYLSLLOVA  
 |||||  
 HUMPN1A FAGKPYECVNTTSGSRFPTSQVANRSECFALMNVSGNVRWKLKVNFDNVGLGYLSLLOVA  
 HUMPN1B FAGKPYECINTTSGSRFPASQVNRSECFALMNVSGNVRWKLKVNFDNVGLGYLSLLOVA  
 HUMPN1C FAGKPYEC -NTTDSRFP -SQV -NRSECFALMNVSG -NVRWKLKVNFDNVGLGYLSLLOVA  
 HUMPN1D FAGKPYECVNTTSGSRFPTSQVANRSECFALMNVSGNVRWKLKVNFDNVGLGYLSLLOVA

RATPN1 1402 TFGKWMIMYAAVDSVNVNEQPKYEYS/LMYIYFVPIIPGSEFTLNLFGIVIDNFNQK  
 |||||  
 HUMPN1A TFGKWMIMYAAVDSVNVNEQPKYEYS/LMYIYFVPIIPGSEFTLNLFGIVIDNFNQK  
 HUMPN1B TFGKWTIIMYAAVDSVNVNEQPKYEYS/LMYIYFVPIIPGSEFTLNLFGIVIDNFNQK  
 HUMPN1C TFGKWTIIMYAAVDSVNV - - -OPKYEYS/LMYIYFVPIIPGSEFTLNLFGIVIDNFNQK  
 HUMPN1D TFGKWMIMYAAVDSVNVNEQPKYEYS/LMYIYFVPIIPGSEFTLNLFGIVIDNFNQK

RATPN1 1463 KKLGGQDIPTHEQKKYNNAMKLGSKKPKQPIPRGNKFGQCFDLVTNQAFDITIMVLI  
 |||||  
 HUMPN1A KKLGGQDIPTHEQKKYNNAMKLGSKKPKQPIPRGNKFGQCFDLVTNQAFDITIMVLI  
 HUMPN1B KKLGGQDIPTHEQKKYNNAMKLGSKKPKQPIPRGNKFGQCFDLVTNQAFDITIMVLI  
 HUMPN1C KKLGGQDIPTHEQKKYNNAMKLGSKKPKQPIPRGNK -GGC FDLVTNQAFDITIMVLI  
 HUMPN1D KKLGGQDIPTHEQKKYNNAMKLGSKKPKQPIPRGNKFGQCFDLVTNQAFDITIMVLI

RATPN1 1524 CLNMVTMVEKEGGQTEYMDVLNWINVFIILPTGECVLKLSLRHYPTVGNWIIYFVVV  
 |||||  
 HUMPN1A CLNMVTMVEKEGGQXKXKXVLKWINVFIILPTGECVLKLSLRHYPTVGNWIKKFFVVV  
 HUMPN1B CLNMVTMVEKEGGQSHTEYLVYNVFIILPTGECVLKLSLRHYPTVGNWIIDPFFVV  
 HUMPN1C CLNMVTMVEKEGGQ - - -H -VL -WIN -VFIIILPTGECVLKLSLRHYPTVGNWII - FVVV  
 HUMPN1D CLNMVTMVEKEGGQTEYMDVLNWINVFIILPTGECVLKLSLRHYPTVGNWIIYFVVV

RATPN1 1585 ILSIVGMFLAEMIEKYFVSTPLFRVIRLARIGRILRLKGAQIRTLFALAMS/LPALFNI  
 |||||  
 HUMPN1A ILSIVGMFLAKXIEKYFVSTPLFRVIRLARIGRILRLKGAQIRTLFALAMS/LPALFNI  
 HUMPN1B ILSIVGMFLADLIETVFSPTLFRVIRLARIGRILRLKGAQIRTLFALAMS/LPALFNI  
 HUMPN1C I-SIVGMFLA - -IE -YFVSPTLFRVIRLARIGRILRL -KGAQIRTLFALAMS/LPALFNI  
 HUMPN1D ILSIVGMFLAEMIEKYFVSTPLFRVIRLARIGRILRLKGAQIRTLFALAMS/LPALFNI

RATPN1 1646 GLLFLVMFIYAI PGMSNFAVYKKEAGINDMFNFETPGNSNICLPQITTSAGWDGLLAPIL  
 |||||  
 HUMPN1A GLLFLVMFIYAI PGMSNFAVYKKEAGINDMFNFETPGNSNICLPQITTSAGWDGLLAPIL  
 HUMPN1B GLLFLVMFIYAI PGMSNFAVYKKEAGINDMFNFETPGNSNICLPQITTSAGWDGLLAPIL  
 HUMPN1C GLLFLVMFIYAI PGMSNFAVYKKE -GINDMFNFETPGNSNICLPQITTSAGWDGLLAPIL  
 HUMPN1D GLLFLVMFIYAI PGMSNFAVYKKEAGINDMFNFETPGNSNICLPQITTSAGWDGLLAPIL

RATPN1 1707 NSAPFDCDPKKVHPGSSVEGDGMPGSGVGIPTFVSYIIISFLVVMYIAVILENFSVATEE  
 |||||  
 HUMPN1A NSAPFDCDPKKVHPGSSVEGDGMPGSGVGIPTFVSYIIISFLVVMYIAVILENFSVATEE  
 HUMPN1B NSAPFDCDPKKVHPGSSVEGDGMPGSGVGIPTFVSYIIISFLVVMYIAVILENFSVATEE  
 HUMPN1C NS -PFDCDPKKVHPGSSVEGDGMPGSGVGIPTFVSYIIISFLVVMYIAVILENFSVATEE  
 HUMPN1D NSAPFDCDPKKVHPGSSVEGDGMPGSGVGIPTFVSYIIISFLVVMYIAVILENFSVATEE

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RATFN1 1768 STEPL DFEMFYEVWEKFPDDATQFIEFKLSDFAAALDPPLLIAK .KVQLIAMDLPM
|||||
HUMFN1A STEPLSEDDDFEMFYEVWEKFPDDATQFIEFKLSDFAAALDPPLLIAKPNKVQLIAMDLPM
HUMFN1B STEPLSEDDDFEMFYEVWEKFPDDATQFIEFKLSDFAAALDPPLLIAKPNKVQLIAMDLPM
HUMFN1C STEPLSEDDDFEMFYEVWEKFPDDATQFIEF - KLSDFAAALDPPLLIAKPNKVQLIAMDLPM
HUMFN1D STEPLSEDDDFEMFYEVWEKFPDDATQFIEFKLSDFAAALDPPLLIAKPNKVQLIAMDLPM

RATFN1 1829 VSGDRIHCLDILFAFTKRVLGEGGEMDSLRQSOMEERFMSANPSKVSYEPITTTLLKRKQEEV
|||||
HUMFN1A VSGDRIHCLDILFAFTKRVLGEGGEMDSLRQSOMEERFMSANPSKVSYEPITTTLLKRKQEEV
HUMFN1B VSGDRIHCLDILFAFTKRVLGEGGEMDSLRQSOMEERFMSANPSKVSYEPITTTLLKRKQEEV
HUMFN1C VSGDRIHCLDILFAFTKRVLGEGGEMDSLRQSOMEERFMSANPSKVSYEPITTTLLKRKQEEV
HUMFN1D VSGDRIHCLDILFAFTKRVLGEGGEMDSLRQSOMEERFMSANPSKVSYEPITTTLLKRKQEEV

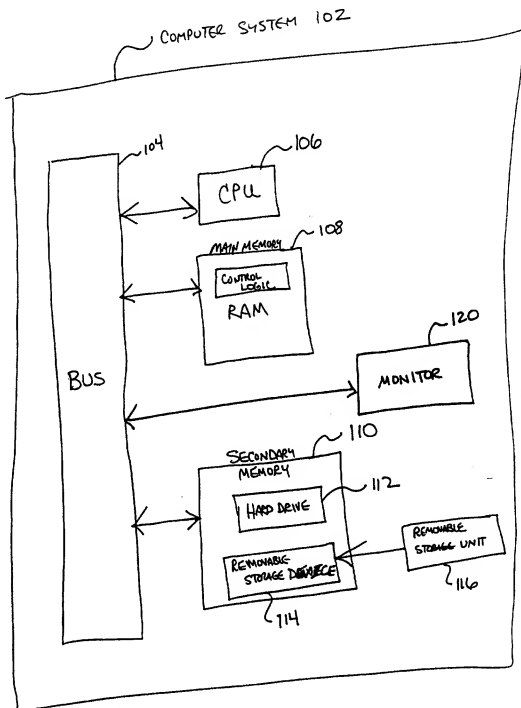
RATFN1 1890 SATIIQRAYRRYRLRQHVKNISSIYIKDGDREDDLPNKEDTVFQNVNENSSPEKTDVTAST
|||||
HUMFN1A SATIIQRAYRRYRLRQHVKNISSIYIKDGDREDDLPNKEDTVFQNVNENSSPEKTDXTXST
HUMFN1B SATVIQRAYRRYRLRQNVKNISSIYIKDGDREDDLLNKKDMAFQNVNENSSPEKTDATST
HUMFN1C SAT - IQRAYRRYRLRQ - VKNISSIYIKDGDREDDLPNKEDTVFQNVNENSSPEKTD - T - ST
HUMFN1D SATIIQRAYRRYRLRQHVKNISSIYIKDGDREDDLPNKEDTVFQNVNENSSPEKTDVTAST

RATFN1 1951 ISPPSYDSVTKPDQEKYETDKTEKEDKEK ESRK- 1985
|||||
HUMFN1A XSPPSYDSVTKPDKEKYEDXTEKEDKXKDSKESKX
HUMFN1B TSPPSYDSVTKPDKEKYEQDRTEKEDKXKDSKESK-
HUMFN1C -SPPSYDSVTKPD - EKYE - D - TEKEDK - KDSKES - K-
HUMFN1D ISPPSYDSVTKPDQEKYETDKTEKEDKEXXESREX

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FIGURE 11D

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FIG. 12  
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CTCTTATGTTG	AGGAGI	AA	GAGGAATTAA	AATATACAGG	ATGAAAAGAT	50
GGCAATGTTG	CCTCCCCCAG		GAGCTCAGAG	CTTTGTCCAT	ATCAAAAAC	100
AGTCTCTTGC	CTCATTAGAA		CACCGATTG	CTGAAAAGAA	ATCAAAAGAA	150
CCCAAGAGAG	AAAGAAGAAG		TGATGATGAA	GAAGCCCCAA	AGCCAGAGAG	200
TGACTTGGAA	GCTGGCAAAAC		AACCTGCCCT	CATCATATGCT	GCATCTCTC	250
CGCGCATGGT	TGACGAGCCCC		CTGAGAGACT	TGAGCCGCTA	CTATGCACAG	300
AAAAAGACTT	TCATAGATTT		GACAAAGGGG	AAAAAATCT	TCGGTTTCAA	350
TGCCACACCT	GCTTTATATA		TGCTTTCTCC	TTTCAGTCTC	CTAGAAGGAA	400
TATCTATATA	GATTTTAGTA		CACCTCTTAT	TCAGCATGCT	CATCATGTGC	450
ACTATCTGGA	CAAACTGCAT		ATTTATGACC	ATGAATAAAC	CGCCGAGCTG	500
GACCAAAAAT	GTGAGTACA		CTTTTACTGG	AATATATACT	TTTGAATCAC	550
TTGTAAAAAT	CTTGCAGAA		GGCTTCTGTG	TAGGAGAATT	CACCTTTCTT	600
CGTGAACCGT	GGAACTGGCT		GGATTTTGTG	GTCACTGTGT	TTGGCTATTT	650
AACAGAAATT	GTAACCTTAG		GCAATGTTTC	AGCTCTTCCA	ACTTTTCAGAG	700
TATTGAGAGC	TTTGAANAAT		ATTTCTGTAA	TCCCAGGCCT	GAAGACAATT	750
TGAGGGGCTT	TGATCCAGTC		AGTGAAGAAG	CTTTCTGATG	TCATGATCCT	800
GACTGTGTTT	TGCTGTAGTG		TGTTTGCACT	AATTGGAGTA	CAGCTGTTCA	850
TGGGAACCTT	GAAGCATAAA		TGTTTTGGA	ATTCACCTGA	AAATAATGAA	900
ACATTTAGAAA	GCATATGAAA		TACCTTAGAG	ATGGAAGAAG	ACTTTAGAAA	950
ATATTTTTAT	TACTTGGAA		GATCCAAAGA	TGCTCTCCTT	TGTGGTTTCA	1000
GACAGAGATTC	AGGTGAGTGT		CCAGAGGGGT	ACACCTGTGT	GAATAATTGC	1050
AGAAACCGCT	ATATGGCTA		CAGAGGCTTT	GACACTTTCA	GCTGGGCTTT	1100
CTTAGCGCTG	TTTAGGCTAA		TGACCCCAAG	TTATCTGGAA	ACTTGGGCTT	1150
AACACAGCTG	CGGTGCTGCT		GGCAAAACTT	ACAGATCTTT	CTTTGTCTTA	1200
GTGATTTTCC	TGCTGCTCTT		TTATCTAATA	AACCTGATCC	TGGCTGTGGT	1250
TGCCATGGCA	TGTAAGAAGC		AGAACCAGCG	AAACATTGAA	GAAGCTAAAC	1300
AGAAAGATTT	AGATTTTCAA		CAGATGTTAG	ACCGCTTAA	AAAGAGAGCAA	1350
GAAGAGCTGT	AGGCATTCTG		AGCGGCAGCG	GCTGAATATA	CAAGTATTAG	1400
GAAGAGCAGA	ATTATGGGCC		TCTCAGAGAG	TTCTTCTGAA	ACATCAAAAC	1450
TGAGCTCTAA	AGTGTCTAAA		GAAGAAGAAA	ACAGAAGAAA	GAAGAAGAAAT	1500
CAAAAGAAGC	TCTCCAGTGG		AGAGGAAGAAG	GGAGATGCTG	AGAAATTGTC	1550
GAATCAGAA	TCAGAGGACA		GCATCAGAG	AAAAAGTTTC	CACCTTGGTG	1600
TCGAAGGGCA	TAGGCGAGCA		CATGAAAAGA	GOTTGTCTAC	CCCCAATCAG	1650
TCACCACCTA	GCATTGCTGG		CTCCTTGTTT	TCGTCAGGCG	GAAGCAGCAG	1700
AACAAGTCTT	TTTAGTTTCA		AGGCGCAGAG	AGAGATATA	GGATCTGAGA	1750
CTGAATTTGC	CGATGATGAG		CACAGCATT	TTGGAGACAA	TGAGAGCAGA	1800
AGGGGCTCAC	TGTTTGTGCC		CCACAGACCC	CAGGAGCGAC	CGAGCAGTAA	1850
CATCAGCCAA	GCCAGTAGGT		CCCCACCAAT	GCTGCGGGTG	AACGGGAAAA	1900
TGCACAGTGC	TGTGGACTGC		AACGGTGTGG	TCTCCCTGGT	TGATGAGCCG	1950
TCAGCCCTCA	TGCTCCCCAA		TGGACAGCTT	CTGCGAGAGG	GCCAGCAAAA	2000
TCAAATACAC	AGCAAAAAGC		GTTGTAGTTC	CTATCTCTCT	TCAGAGATTA	2050
TGCTGAATGA	TCCCACCTTC		AGACAGAGAG	CAATGAGTGA	AGCAGACATA	2100
TTAACCAACA	CTGTGGGAAG		ACTTGAAGAG	TCCAGACAAA	AATGTCACCC	2150
TTGTGGTATC	AGATTGTGAC		ACAAATTTCT	GATCTGGAAAT	TGCTCTCCAT	2200
ATTGGATAAA	CAATTACCAT		TTGCAATGTT	TTAAACACAT	TAITTTATGCG	2250
GTAGATCTTG	CACCCAAATG		CTGAGGAATT	CAAAAATGTA	CTGTCTATAG	2300
GAATTTGTGT	CTTTACTGGA		ACTTTTGAG	CTGAAATGGT	ATTAAACATG	2400
ATTGCCATGG	ATCCATATGA		GTATTTCCAA	GTAGGCTGGA	ATATTTTTGA	2450
CAGCCTTATT	GTGACTTTAA		TTTGTAGTGA	GCTCTTTCTA	GCAGATGTGG	2500
AAGGATTGTC	AGTTCGTGGA		TCATTAGAC	TGCTCCGAGT	CTTCAAGTTG	2550
GCAAAATCTC	GGCCAACTAT		GAACATGCTG	ATTAGATCAT	TTGTAACTC	2600
AGTAGGGGCT	CTAGGTAACC		TCAACCTTAGT	GTGGGCCATC	ATGCTCTTCA	2650
TTTTTGCTGT	GGTGGGCTAT		CAGCTCTTTG	GTAAAGAGCTA	CAAGAATGTT	2700
GTCTGCAAGA	TCAATGATGA		CTGTAGCTCT	CCACGGTGGC	ACATGAACGA	2750
CTTCTTCCAC	TCTCTCTGTA		TTGTGTTCCG	GCTGCTGTGT	GGAGAGTGA	2800
TAGAGACCAT	GTGGGACTGT		ATGGAGGTCC	CTGGTCAAGC	TATGTGCTT	2850
ATTGTTTACA	TGATGTGTCAT		GGTCAATTGA	AACCTGGTGG	TCTTAACTC	2900
ATTCTTGGCC	TTATTATTGA		GCTCAATTAG	TTACAGCAAT	CTTACAGCAA	2950
TTAGAGAAGA	CCCTGATGCA		AACCAACCTCC	AGATTGCAAT	GACTAGAAAT	3000
AAAAAGGGAA	TAAATATTGT		GAAGCAACCC	TTAGCTGAAT	TTATTCTAAA	3050
AGCATTTTTC	AAAAAGCCAA		AGATTTCCAG	GGAGTAAAGA	CAAGCAGAGAG	3100
ATCTGAATAC	TAGAGAGGAA		AACTATATTT	CTAACCATAC	ACTTGTCTGAA	3150
ATGAGCAAGG	GTGACAAATT		CCTCAAGGAA	AAAGTAAAAA	TCAGTGGTTT	3200
TTATTCACAA	TCCACAGCTC		ACAGTGCACG	TGCCAATTGC	ACCTGGGGGA	3250
TCCGATTTGG	AAATATGAAA		TGCTGAGGAA	CTTAGCAGTG	ATTCGGATAG	3300
TGAATCAGCG	AAAGTGAAT		TAAACCGGCT	AGCTCTCTCA	GAGTGACGCA	3400

FIGURE 13A

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CAGTTGATAA	CCCTTTCCT	GGAGAAGGAG	AAGAAGCAGA	GCTGAACTCT	3450
ATGAATTCCG	ATGAGCCAGA	GCCCTTTTTC	ACAGATGGTT	GTGTACCGAG	3500
GTCTCTATCG	TGCGAAGTTA	ACATAGAGTC	AGGGAAGAGA	AAATCTGGT	3550
GGACATACAG	GAAACCTCG	TACAAGATTG	TTGAACACAG	TGTGTTTGAA	3600
AGCTTCATTG	TCTCATGAT	CCTGCTCAGC	AGTGGTGCCC	TGCGTTTGA	3650
AGATATTAT	ATTGAAGGA	AAAAGCCAT	TAAGATTATC	CTGGAGTATG	3700
CAGACAAGAT	CTTCACTTAC	ATCTTCATTC	TGGAAATGCT	TCTAAATGG	3750
ATAGCATATG	GTATAAAAC	ATATTTCACC	AATGCCCTGG	GTGGCTGGGA	3800
TTTCTTAATT	GTGTAGTATT	CTTTGGTTAC	TTTAGTGGCA	AACACTCTTG	3850
GCTACTCAGA	TCTTGGCCCC	ATTAAATCCC	TTGGGCACT	GAGAGCTTTA	3900
AGACCTCTAA	GAGCCTTATC	TAGATTGAA	GGATGAGGG	TGTTGTGAA	3950
TGCACCTATA	GGAGCAATTC	CTTCCATCAT	GAATGTGCTA	CTTGTGTGTC	4000
TTATATTCTG	GTGATATTCT	AGCATCATGG	GAGTAATTTT	GTTTGCTGGC	4050
AAGTTCATAT	AGTGTATTAA	CACCACAGAT	GGGTACGGT	TTCTGTCAG	4100
TCAAGTCCAA	AATGGTTCOG	AATGTTTTGC	CCTTATGAAT	GTTAGTCAAA	4150
ATGTGCGATG	GAAAAACCTG	AAAGTGAAT	TTGATAATGT	CGAGCTTGGT	4200
TACCTATCTC	TGCTTCAGT	TGCACTTTT	AGGGATGGA	CGATTATTAT	4250
GTATGCGACA	GTGGATTCTG	TTATGTGAGA	CAAGCAGCCC	AATATGAAT	4300
ATAGCCTCTA	CATGTATATT	TTATTTGTGC	TCTTTATCAT	CTTTGGGTCA	4350
TTCTTCACTT	TGAATCTGTT	CATTGGTGTG	ATCATAGATA	ATTTCAACCA	4400
ACGGAAGAA	AGCTCTGGAG	GTCAAGACAT	CTTTATGACA	GAAGAACAGA	4450
AGAAATCTA	TATGCAATG	AAAAAGCTGG	GGTCCAGAA	GCCACAAGAG	4500
CCAATCTCTC	GACCAAGGGA	CAAAATCCAA	GGATGTATAT	TTGACTAGT	4550
GACAATCAAA	GCCTTTGATA	TTAGTATCAT	GGTTCTTATC	TGTCTCAACA	4600
TGGTAACCAT	GATGGTAGAA	AAGGAGGGTG	AAAGTCAACA	TATGACTGAA	4650
GTTTTATATT	GGATAAATGT	GGTTTTTATA	ATCCTTTTCA	CTGGAGAAATG	4700
TGTGCTAAAA	CTGACTCCCC	TGAGACACTA	CTACTCACT	GTAGAGTGA	4750
ATATTTTTGA	TTTTGTGGTT	GTGATTATCT	CAATTGTAGG	TATGTTTCTA	4800
GCTGATTGA	TTGAACGTA	TTTTGTGTCC	CCTACCTGTT	TCCGAGTATG	4850
CGTCTTGCC	AGGATTGGCC	GAATCCTAGC	TCTAGTCAAA	GGAGCAAGG	4900
GGATCGGCAC	GCTGCTCTTT	GCTTGTGATG	TGTCCCTTCC	TGGTGTGTTT	4950
AACATCGGCC	TCTGCTCTTT	CCTGGTCATG	TTCACTACG	CCATCTTTGG	5000
AATGTCCAAC	TTTGCTGATT	TTAAAAAGGA	AGATGGAATT	AATGACATGT	5050
TCAATTTTGA	GACCTTTGGC	AACAGTATGA	TTTGGCTGTT	CCAAATAGA	5100
ACCTCTGCTG	GCTGGGATGG	ATTGTGACGA	CTTATCTTTA	ACAGTAAAGC	5150
ACCCGACTGT	GACCCAAAAA	AAAGTTTATCC	ACTTGTGTAG	GTGGAAGGAG	5200
ACTGTGGTAA	CCCATCTGTT	GGAATATTCT	ACTTGTGTAG	TTATATCATC	5250
ATATCCTTCC	TGGTGTGGT	GAAATCTATC	ATTCAGTCA	TACTGGAGAA	5300
TTTAGTGTTT	GCCACTGAGG	AAAGTACTGA	ACCTCTGAGT	GAGGATGACT	5350
TTTATAGAGT	CTATGAGGTT	TGGGGAAGT	TTGATCCCGA	TGCGACCCAG	5400
TCTTCTCATA	TCTCTAAACT	CTCTGATTTT	GCAGCTGCCC	TGGATCCTCC	5450
CCATGGTTAG	TGGTGCACGG	ATCCATTGTC	TTGACATCTT	ATTGCTTTT	5500
ACAAAGCGTG	TTTTGGGTGA	GAGTGGGGAG	ATGGATTCTC	TTGTTTCAAC	5550
GATGGAAGAA	AGGTTTATGT	CTGCAAAATC	TTCCAAAGTG	TCCTATGAAC	5600
CCATCAACA	CACACTAAAA	CGGAACAGAG	AGGATGTGTC	TGCTACTGTC	5650
ATTGAGCGTG	CTTATAGAGC	TTACCGCTTA	AGGCAAAATG	TCAAAATAT	5700
ATCAAGTATA	TACATAAAG	ATGGAGCAG	AGATGATGAT	TTACTCAATA	5750
AAAAAGATAT	GGCTTTTGAT	AATGTTAAAT	AGAACTCAAG	TCCAGAAAAA	5800
ACAGATGCCA	CTTCACTCAC	CACCTCTCCA	CCTTTCATATG	ATAGTGTAAAC	5850
AAAGCCAGAC	AAAGAGAAAT	ATGAACAGA	CAGAACAGAA	AAGGAGACA	5900
AAAGGGAAGA	CAGCAAGGAA	AGCAAAAAAT	AGAGCTTCAT	TTTGTGATA	5950
TGTTTACAG	CCGTGTGAAG	TGATTATTAT	GTGTTAATAA	AACTCTTTTG	6000
AGGAAGCTTA	TGCCAAATTC	CTTTTATGCA	CTAGAAAGG	TGGGAGCAT	6050
CAGTCACTAA	CTCTGATTTC	CTTAGAGAA	GGTAAAGGAA	CTCTGTAGGA	6100
TTATTTTTTC	ACTGATGAT	CTTAGAGAA	GGTAAAGGAA	CTCTGTAGGA	6150
ATATTATGAT	ATGACATACA	AAAGTGATTG	ATTCAGTTTT	TGTTGTTTTA	6200
ATAAATCAGA	AGACCATGTA	GAAAACCTTT	ACATCTGCTT	TGTCATCTTT	6250
TCACAGGAT	GTAATTAGTC	TTGTTTCCCA	TGTAATAAAA	CAACACAGC	6300
ATACAGAAAA	AAAAAAAAAA	A			6371

FIGURE 13B

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CTCTTATGTG	AGGAGCAGAA	GAGGAATTAA	ATATACAGG	ATGAAAGAT	50
GGCAATGTTG	CCTCCCCAG	GACCTCAGAG	CTTTGTCCAT	TTCAAAAC	100
AGTCTCTTGC	CCTCATTGAA	CAACGCATTG	CTGAAAGAA	ATCAAGGAA	150
CCCAAGAGAG	AAAGAAGAA	TGATGATGAA	GAGGCCCAAG	AGCCAGCAG	200
TGACTTGTGAA	CTGTGCAAC	AACCTGCTTT	CATCTATGGG	GACATCTCTC	250
CGGCGATGGT	GTGACAGGCC	CTGAGAGACT	TGGACCCCTA	CTATGCAAG	300
AAAGAAGACT	TCATAGTATT	GAACAAAGG	AAACAATCT	TCCGTTTCAA	350
TGCCACACCT	GCTTTATATA	TGCTTTCTCC	TTTCAGTCCT	CTAGAAGAA	400
TATCTATTAA	GATTTTAGTA	CACCTCTTAT	TCAGCATGCT	CATCATGTGC	450
ACTATTCTGA	CAAACTGCAT	ATTTATGACC	ATGAATAACC	CGCCGCACTG	500
GACCAAAAAT	GTGAGTACA	CTTTTACTGG	AATATATACT	TTTGAATCAC	550
TTGTAAAAAT	CCTTGCAAGA	GGCTTCTGTG	TAGGAGAATT	CACCTTTTCT	600
CGTGAACCGT	GGAACTGGCT	GGATTTTGTG	GTCAATGTTT	TGTGTAATTT	650
AACAGAAATT	GTAACACCTAG	GCAATGTTTC	AGCTCTTCGA	ACTTTTCAGAG	700
TATTGAGAGC	TTTGAANAAT	ATTTCTGTAA	TCCAGGCCT	GAAGAATTT	750
GTAGGGGCTT	TGATCCAGCT	AGTGAAGAG	CTTTCTGATG	TGATGATCT	800
GACTGTGTTG	TGTCTGAGTG	TGTTTGCAT	AATTGGACTA	CAGCTGTTCA	850
TGGGAACCTT	GAAGCATAAA	TGTTTTGAAA	ATTCACCTGA	AAATAATGAA	900
ACATTAGAAA	GCTAATGAAA	TACCTTAGAG	AGTGAAGAG	ACTTTAGAAA	950
ATATTTTTAT	TAGTTGGAAG	GATCCAAAG	TGCTTCTCTT	TGTGTTTCA	1000
GCACAGATTC	AGGTGAGTGT	CCAGAGGGGT	ACACCTGTGT	GAAATTTGCT	1050
AGAAACCCGT	ATATGTGCTA	CACAGCTTTT	GACATTTCA	GCTGGGCTT	1100
CTTAGGCTTG	TTTAGGCTAA	TGACCCAGA	TACTGGGA	AACCTTTACC	1150
AACAGAGCTT	GCCTGCTGCT	GGCAAAACCT	ACATGATCTT	CTTTGTGCTA	1200
GTGATTTGCT	TGGCTCTCTT	TTATCTAATA	AACCTGATCC	TGGCTGTGCT	1250
TGCCAGGCA	TATGAAGAAG	AGAACCAGGC	AAACATTGAA	GAAGCTAAAC	1300
AGAAGAATTT	AGAAATTTCA	CAGATTTTGA	ACCGCTTAA	AAAAGAGCAA	1350
GAAGAAGCTG	AGCAATTTGC	AGCGGCAGCG	GCTGAATATA	CAAGTATTAG	1400
GAGAAGCAGA	ATTATGGGCT	TCTCAGAGAG	TTCTTCTGAA	ACATCCAAAC	1450
TGAGCTCTAA	AGTGCTAATA	GAAGAAGAA	ACAGAAGAA	GAAGAAGAA	1500
CAAAAGAGC	TCTCCAGTGC	AGAGGAAGAG	GGAGTGTCTG	AGAAATTTGT	1550
GAAATCAGAA	TCAGAGGACA	GCATCAGAG	AAAAGTTTCT	CACCTTGTGT	1600
TGGAAGGGCA	TAGGCGAGCA	CATGAAAGAA	GTTTGTCTAC	CCCCAATCAG	1650
TCCACACTCA	GCATTTGCTG	CTCCTTGTTT	TCTGCAAGGC	GAAGCAGCAG	1700
AACAAGTCTT	TTTAGTTTCA	AAGGCAGAGG	AAAGATATA	GGATCTGAGA	1750
CTGAATTTGC	CGATGATGAG	CACAGCATT	TTGAGACAA	TGAGAGCAGA	1800
AGGGGCTCAC	TGTTTGTGCT	CCACAGACCC	CAGAGCGCAC	CGAGCAGTAA	1850
CATCAGCCAA	GCAGTAGGT	CCCCCAAT	GCTGCGGTG	AACGCGAATA	1900
TGCAAGTGTG	TGTGACTGCT	AACGGTGTG	TCTCCCTGCT	TGATGAGCC	1950
TGCGGCTCA	TGCTCCCAAC	TGGACAGCTT	CTCCCGAGG	TGATATAGA	2000
TAGACAACT	TCTGATGACA	CGCGACAC	GAATCAATA	CACAGAAATA	2050
GGGTTGTGAT	TTCTATCTC	CTTCTCAGG	ATATGCTGAA	TGATCCCAAC	2100
CTCAGACAGA	TGCTATAGAG	TAGAGCAAGC	ATATTAACAA	ACACTGTGGA	2150
AGAACTTGA	GAGTCAGAG	AAATATGCC	ACCTTGTGTT	TACAGATTGG	2200
CACAGAAAT	CTTGTCTG	AATTGCTCTC	CATATTGGAT	AAATTTCAA	2250
AAAGTATCT	ATTTTATTGT	AATGATCTCT	TTTGTAGATC	TTGCAATTAC	2300
CATTGTGATA	GTTTTAAACA	CATTATTAT	GGCTATGGA	CACCAACCAA	2350
TGACTGAGGA	ATTCAAAAT	GTACTTGCTA	TAGGAATTT	GGTCTTTACT	2400
GGAACTTTTG	CAGCTGAAAT	GGTATTAAAA	CTGATTGCCA	TGGATCCATA	2450
TGAGTATTTT	CAGTAGGCT	GGATATTTT	TGACAGCTT	ATTGTGACTT	2500
TAGTTTGTAGT	GGAGCTCTT	CTAGCAGATG	TGGAAAGATT	GTCAAGTTCTG	2550
CGATCATTTA	GACTGCTCCG	AGTCTTCAAG	TTGGCAAAAT	CCTGGCCAAC	2600
ATTGAACATG	CTGATTAAAG	TCAATTGTTAA	CTCAGTAGGG	GCTCTAGGTA	2650
ACCTCACCTT	AGTGTGGGCC	ATCATGCTCT	TCAATTTTGC	TGTGTGCGG	2700
ATGACGCTCT	TTGTGAAGAG	CTACAAAGAA	TGTGTCTGCA	AGATCAATGA	2750
TGACTGTAGC	TGCCACGGT	GGCAATGAA	GAATCTCTTC	CATCTCTTCC	2800
TGATTTGTGT	CGCGGTGCTG	TGTGAGAGT	GGATAGAGAC	CATGTGGAC	2850
TGTATGTGAGG	TGCTGAGTCA	AGCTATGAA	CTTATGTTT	ACATGATGCT	2900
CATGTTGATT	GGAAACCTGG	TGCTCTTAA	CTTATTTCTG	GCCTATTAT	2950
TGAGTCTATT	TAGTTCAAG	AATCTTCAAG	CAATTAAGAA	AGACCTGTAT	3000
GCAAACCACT	TGAGATGAG	AGTCACTAGA	ATTAAAGAGG	GAATAATTA	3050
TGTGAACAAA	ACCTTACGTT	AATTTATCT	AAAAGCAAT	TGCAAAAGC	3100
CAAGATTTTC	CAGGAGATA	AGACAGCAG	AAGATCTGAA	TACTAAGAG	3150
GAAGACTATA	TTTCTAACCA	TACACTTGTCT	GAATAGACAA	AGGTGACAA	3200
TTTCTCTAAG	GAAGAGATA	AAATCAGTGG	TTTGTGAAGC	AGGCTGGACA	3250
AACACTTGAT	GGAGACAGT	GATGCTCAAT	CATTATTCCA	CAATCCAGC	3300
CTCAGCTGTA	CAGTGCCAAT	TGACACTGGG	GAATCCGATT	TGGAATATTA	3350
GAATGCTGAG	GAATCTAGCA	GTGATTCGGA	TAGTGAATAC	AGCAAGATGA	3400

FIGURE 14A

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GATTAACCG	GTCAAG..CC	TCAGAGTCCA	GCACAGTTGA	TAACCCCTTG	3450
CCTGGAGAG	GAGAAGAGC	AGAGGCTGAA	CCTATGAATT	CCGATGAGCC	3500
AGAGGCCGT	TTCAAGAGT	GTGTGTACG	GAGGTTCTCA	TGCTGCCAAG	3550
TTAACATAGA	GTCAAGGAAA	GGAAAAATCT	GGTGGAACAT	CAGGAAAAAC	3600
TGCTACAGA	TTGTTGAACA	CAGTTGGTTT	GAAGACTTCA	TTGTCTCAT	3650
GTCTCTGCTC	AGCAATGGTG	CCCTGGCTTT	TGAAGATATT	TATATTGAAA	3700
GGAAAAAGAC	CATTAGAATT	ATCTCGAGT	ATGCAGACAA	GATCTTCACT	3750
TACATCTTCA	TTCTGGAATT	GCTTCTAAAA	TGGATAGCAT	ATGGTTATAA	3800
AACATATTC	ACCAATGCCT	GGTGTGGCT	GGATTTCTTA	ATTGTTGATG	3850
TTTCTTTGGT	TACTTTAGTG	GCAACACTC	TTGGCTACTC	AGATCTTGGC	3900
CCCATTAAT	CCCTTCGGAG	ACTGAGAGCT	TTAAGACCTC	TAAGAGCCTT	3950
ATCTAGATTT	GAAGGAATGA	GGGTGGTTGT	GAATGCACAT	ATAGGAGCAA	4000
TTCTCTTCAT	CATGAATGTG	CTACTTTGTG	GTCTTATATT	CTGGCTGATA	4050
TTGAGCATCA	TGGGAGTAAA	TTGTTTGTCT	GGCAAGTTCT	ATGAGTGTAT	4100
TAACACCACA	GATGGGTCAC	GGTTTCTGCG	AAGTCAAGTT	CCAAATCGTT	4150
CCGAATGTTT	TGCCCTTATG	AATGTTAGTC	AAATGTGGG	ATGGAAAAAC	4200
CTGAAGTGA	ACTTTGATAA	TGTCGAGCTT	GTTTACTCAT	CTCTGCTTCA	4250
AGTTGCAACT	TTTAAGGGAT	GGACAATTAT	TATGTATGCA	CGAGTGGATT	4300
CTGTTAATGT	AGACAAGCAG	CCCAAAATAG	AAATATAGCT	CTACATGTAT	4350
ATTTAATTGT	TGCTCTTTAT	CATCTTTGGG	TCATTCTTCA	CTTTGAACTT	4400
GTTCATTGGT	GTCACTATAG	GTCACTATAG	ATAATTTCAA	CCACAGAA	4450
GAGGTCAAGA	CATCTTTATG	ACGAAGAGAC	AGAAGAATA	AGAGAGCTTG	4500
ATAAAAAGC	TGGGTGCAAA	GAAGCCACAA	AAGCCAATTC	CTGACCCAGG	4550
GAACAATACT	CAAGGATGTA	TATTTGACCT	AGTGACAAAT	CAAGCCTTTG	4600
ATATTAGTAT	CATGGTTCTT	ATCTGTCTCA	ACATGGTAAC	CATGATGGTA	4650
GAAGAAGGAG	GTCAAAATCA	ACATATGACT	GAAGTTTTAT	ATTGGATAAA	4700
TGTGGTTTTT	ATAATCCTTT	TCAGTGGAGA	ATGTGTGCTA	AAACTGATCT	4750
CCCTCAGACA	CTACTACTTC	ACTGTAGGAT	GGATATTTTT	TGATTTTGTG	4800
GTGTGATTA	TCTCCATTGT	AGGTATGTTT	CTAGCTGATT	TGATTTGAAAC	4850
GTATTTTGTG	TCCCTACCC	TGTTCCGAGT	GATCCGTCTT	GCCAGGATTG	4900
GCGAATCTCT	ACGCTTAGTC	AAAGGAGCAA	AGGGGATCCG	CACGCTGCTC	4950
TTTGTCTTGA	TGATGTCCTC	TCTGCGTTG	TTTAACATGC	GCCTCTGCTC	5000
CTTCTCGTCT	ATGTTCACTC	ACGCCATCTT	TGGAATGTCC	AACCTTGCCT	5050
ATGTTAAAAA	GGAAGATGGA	ATTAATGACA	TGTTCAATTT	TGAGACCTTT	5100
GGCAACAGTA	TGATTTGCTC	GTTCCAAATT	ACAACTCTGT	CTGGCTGGGA	5150
TGATTTGCTA	GCACTTATTC	TTAACAGTAA	GCACCCGAC	TGTGACCCAA	5200
AAAAAGTTCA	TCTTGAAGTT	CAGTTGAAG	GGAAGTGTG	GTAAACCAATCT	5250
GTGGGAATAT	TCTACTTTGT	TAGTTATATC	ATCATATCTT	TCTCTGGTTG	5300
GGTGACATGT	TACATTGTCG	TCATATCGGA	GAATTTAGT	GTGCGCACTG	5350
AAGAAGTATC	TGAACCTCTG	AGTGAAGATC	ACTTTGAGAT	GTTCATGAG	5400
GTTTGGGAGA	AGTTTATATC	CCATGCGACC	CAGTTTATAG	AGTTCCTAA	5450
ACTCTCTGAT	TTTGACAGCT	CCCTGGATCC	TGCTCTTCTC	ATAGCAAAAC	5500
CGAACAAGT	CCAGCTCATT	GCCATGGATC	TGCCCATGTG	TAGTGGTGAC	5550
CGATCCATT	GTCTTGACAT	CTTATTGCTT	TTTACAAGC	GTGTTTGGG	5600
TGAGTGTGGG	GAGATGGATT	CTCTTCTTCT	ACAGATGGAA	GAAGGTTTCA	5650
TGTCGTCAAA	TGCTTCCAAA	GTGTCCTATG	AACCCATCAC	AACCACTATA	5700
AAACGGAAAC	AAGAGGATGT	GTCTGCTACT	GTCACTCAGC	GTGCTTATAG	5750
ACGTTACCGC	TTAAGGCATA	ATGTCAAAAA	TATATCAAGT	ATATACATAA	5800
AAGATGGAGA	CAGAGATGAT	GATTTACTCA	ATAAAAAAGA	TATGGCTTTT	5850
GATAATGTTA	ATGGAACATC	AAAGTCAGAA	AAAAAGATG	CCACTTCATC	5900
CACCACTCTC	CCACCTTCAT	ATGATAGTGT	ACAAAGGCCA	GACAAAGAGA	5950
AATATGAACA	AGACAAGACA	GAAGAAGGAG	ACAAAGGGAA	AGACAGCAG	6000
GAAGACAAA	AATAGAGCTT	CATTTTGTAT	ATATTGTTTA	CAGCTGTGAA	6050
AAGTGATTTA	TTGTGTGTTA	TAAAACTCTT	TTGAGGAAGT	CTATGCCAAA	6100
ATCCTTTTTA	TCAAAATATT	CTCGAAGGCA	GTGCAAGTCA	TAACTCTGAT	6150
TTCTTAGAAA	AGGTTGGGCG	CATTAGCAGA	GTGCAAGTCA	TGACATGATG	6200
ATTCCTTAAG	AATCGTAAGA	GAACTCTGTA	GGATTAATG	ATTATAGCAT	6250
ACAAAAGTGA	TTGATGTAGT	TTTGTGGTTT	TTAATAAATC	AGAAAGCCAT	6300
GTAGAAAACT	TTTACATCTG	CCCTGTCATC	TTTTCAAGG	ATTGTATATA	6350
GTCTTGTTC	CCATGTAAAT	AAACAACACA	CGCATACAGA	AAAAAAGAAA	6400
AAAA					6404

FIGURE 14B

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## INTERNATIONAL SEARCH REPORT

 International application No.  
 PCT/US95/14251

## A. CLASSIFICATION OF SUBJECT MATTER

 IPC(6) : A61K 35/00, 31/00; C12N 15/00; G01N 16/00  
 US CL : 536/22.1; 530/350, 387.1; 435/6; 436/86; 514/44;

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/22.1; 530/350, 387.1; 435/6; 436/86; 514/44;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

 Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 APS, MEDLINE, BIOSIS, EMBASE, CAPLUS, WPIDS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO/A, 90/09391 (ARCH DEVELOPMENT CORPORATION) 23 August 1990, see entire document.	1-39
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, Volume 89, issued September 1992, AHMED ET AL., "Primary Structure, Chromosomal Localization, and Functional Expression of a Voltage-Gated Sodium Channel from human Grain", pages 8220-8224, see entire document.	1-39

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understate the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

24 JANUARY 1996

Date of mailing of the international search report

28 FEB 1996

 Name and mailing address of the ISA/US  
 Commissioner of Patents and Trademarks

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## INTERNATIONAL SEARCH REPORT

 International application No.  
 PCT/US95/14251

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DNA AND CELL BIOLOGY, Volume 13, Number 1, Issued 1994, SHENG ET AL., "Molecular Cloning and Functional Analysis of the Promoter of Rat Skeletal Muscle Voltage-Sensitive Sodium Channel Subtype 2 (rSkM2): Evidence for Muscle-Specific Nuclear Protein Binding to the Core Promoter", pages 9-23, see entire document.	1-39
A,P	SCIENCE, Volume 269, Issued 25 August 1995, MARSHALL, "Gene Therapy's Growing Pains", pages 1050-1055, see entire document.	1-5, 10-11, 22-29
Y	BIOPHYSICAL JOURNAL, Volume 66, Issued January 1994, LIPKIND ET AL., "A Structural Model of the Tetrodotoxin and Saxitoxin Binding Site of the Na <sup>+</sup> Channel", pages 1-13, see entire document.	1-39
Y	BIOCHEMISTRY, Volume 31, Issued 1992, WAKAMATSU ET AL., "Structure-Activity Relationships of u-Conotoxin GIIIA: Structure Determination of Active and Inactive Sodium Channel Blocker Peptides by NMR and Simulated Annealing Conditions", pages 12577-12584, see entire document.	6-9, 12-16, 30-39
Y	PROTEIN ENGINEERING, Volume 6, Number 1, Issued 1993, SANSOM ET AL., "Influenza Virus M2 Protein: A Molecular Modeling Study of the Ion Channel", pages 65-74, see entire document.	16-21, 30-39
Y,P	US, A, 5,380,836 (ROGART) 10 January 1995, see entire document.	1-39

# INTERNATIONAL SEARCH REPORT

Int'l application No.  
PCT/US95/14251

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.  
☒ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US95/14251

### BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-5 and 10-11, drawn to isolated nucleic acid molecules and nucleic acid probes.

Group II, claim(s) 6-9, drawn to isolated peptides.

Group III, claim(s) 12, drawn to methods of detection exploiting a nucleic acid.

Group IV, claim(s) 13-16, drawn to antibodies and methods of detection exploiting a peptide.

Group V, claim(s) 17-21, drawn to bioassays for modulating agents.

Group VI, claim(s) 22-29, drawn to methods of treatment through in vivo delivery of a nucleic acid construct.

Group VII, claim(s) 30-39, drawn to methods of providing a molecular model, computer readable mediums, and computer based systems for providing molecular models of a biological ligand.

The inventions listed as Groups I through VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The claims in group I relate to nucleic acid molecules which possess markedly different physical and biochemical characteristics than the peptides of group II. Group III is considered distinct because the claims therein pertain to methods of detection through the use of a nucleic acid. The existence of the nucleic acid molecule does not rely on the methods of its detection in any environment. Group IV pertains to antibodies and methods of detection using said antibodies. The antibodies used in this group possess many different physical and biochemical characteristics from either nucleic acids or peptides. Group V pertains to bioassays which assess a modulating agent of a PNS SCP wherein said bioassay is not essential to either the peptide or nucleic acid as is instantly claimed. Group VI pertains to methods of treatment wherein a nucleic acid molecule is administered in vivo. Many factors must be considered prior to in vivo administration of a polynucleotide, stability and targeting, for example. The claims of group VII pertain to methods for providing a molecular model of a sodium channel peptide, computer readable mediums, and computer based systems for providing molecular models. This group is considered distinct from the previous groups because the existence of a peptide or nucleic acid, or methods of treating using either, do not rely on computer generated models or computer readable mediums.